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**Joint Program
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DOCTORAL DISSERTATION

Migratory Patterns of American Shad
(*Alosa Sapidissima*) Revealed by
Natural Geochemical Tags in Otoliths

by

Benjamin Walther

February 2007

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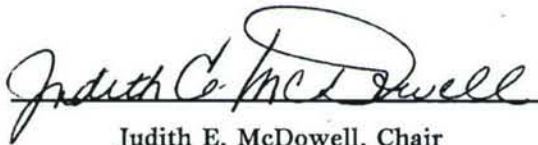
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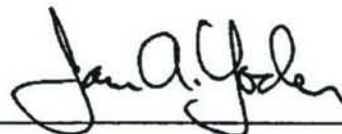
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**MIGRATORY PATTERNS OF AMERICAN SHAD (*ALOSA SAPIDISSIMA*)
REVEALED BY NATURAL GEOCHEMICAL TAGS IN OTOLITHS**

By

Benjamin Walther

B.S. & B.A., University of Texas at Austin, 2000

Submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

and the

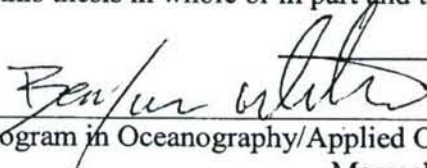
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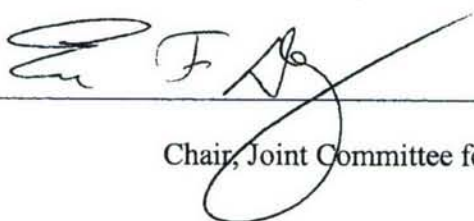
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MIGRATORY PATTERNS OF AMERICAN SHAD (*ALOSA SAPIDISSIMA*) REVEALED BY NATURAL GEOCHEMICAL TAGS IN OTOLITHS

By

Benjamin Walther

Submitted to the MIT Department of Biology and the WHOI Biology Department on
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Doctor of Philosophy

ABSTRACT

Geochemical signatures in the otoliths of diadromous fishes may allow for retrospective analyses of natal origins. In an assessment of river-specific signatures in American shad (*Alosa sapidissima*), an anadromous clupeid native to the Atlantic coast of North America, stable isotope and elemental ratios in otoliths of juvenile American shad produced accurate natal tags from 12 rivers. Significant inter-annual variability in geochemical signatures from several rivers was detected, due largely to differences in $\delta^{18}\text{O}$ values among years. The database was further expanded to include 20 rivers from Florida to Quebec, encompassing all major spawning populations. This task was accomplished by collecting juvenile otoliths along with water samples from rivers where juveniles were not sampled. Regressions between otolith and water chemistry for those rivers where both were collected showed significant relationships for Sr:Ca, Ba:Ca, $\delta^{18}\text{O}$, and $^{87}\text{Sr}:^{86}\text{Sr}$ ratios but not for Mg:Ca or Mn:Ca. Despite reducing the combined signature to only four chemical ratios, cross-validated classification accuracies of known-origin juveniles averaged 93%. Ground-truthed signatures were used to classify migrants of unknown origins. Adults returning to spawn in the York River were classified according to their otolith composition. Only 6% of spawners originated from rivers other than the York, supporting the hypothesis that most American shad spawn in their natal river. Of remaining spawners, 79% originated from the Mattaponi River and 21% from the Pamunkey River. The results suggested that while most American shad home to their natal river there is less fidelity to individual tributaries, allowing subsidies to subpopulations with persistent recruitment failure. Otolith signatures were also used in mixed-stock analyses of immature migrants along the coast of Maine in the spring and Minas Basin in the summer. Mixed-stock compositions showed remarkably low diversity and were dominated by fish from the Shubenacadie and Hudson rivers, with an increasing proportion of Potomac River fish over time. In contrast to results from adult tagging studies, southern stocks were virtually absent. These data suggest ontogenetic shifts in migratory behavior. The thesis concludes with a report that water contributed 83% of Sr and 98% of Ba in the otoliths of a marine fish.

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Chapter 1

INTRODUCTION & BACKGROUND

1.1 MIGRATIONS

Many animal species from divergent taxonomic groups undertake significant migrations during particular phases of their life cycle. Migration, defined as the coordinated, seasonal and usually cyclical movements of the majority of a population, occurs when individuals suppress their sensitivities to stimuli that would otherwise invoke station-keeping behavior and instead move between well-defined geographic areas (Kennedy 1985; Dingle 1996; Dodson 1997). Some terrestrial animals migrate across significant distances, with journeys of thousands of kilometers observed in species including songbirds and butterflies (Chamberlain et al. 1997; Hobson et al. 1999; Rubenstein et al. 2002). Such remarkable movements are not restricted to terrestrial habitats, and seasonal migrations of marine mammals and fishes are well known (Hollis 1948; Killingley 1980; Quinn 1985). Yet though the existence of migratory behavior in certain species has been well documented, detailed knowledge about the routes or destinations of these movements is lacking for the majority of migratory marine animals.

The difficulty in gathering data on large-scale movements of terrestrial and marine organisms lies in the challenges inherent in tracking individuals over sufficient time periods to allow accurate measurements of migratory behaviors. Traditional approaches to tracking individuals involve mark-recapture methods in which recovered tags indicate the direction and destination of migrants (Dingle 1996). Technological

improvements on this approach involve satellite telemetry and digital archiving tags to allow more precise mapping of the routes taken between tagging and recovery sites (Webster et al. 2002; Block et al. 2005). However, artificial tags must be applied without affecting the behavior of the organism or increasing the likelihood of individual mortality (McFarlane 1990). Tagging studies are also effective only if they result in sufficient returns to allow realistic assessments of movement patterns (Schwarz and Arnason 1990). Investigations of fish migrations are particularly sensitive to the issues of invasiveness and the impracticality of tags. This is largely because most fish species are extremely small at birth and do not grow to sizes that can withstand handling until they are much older. As a result, movements during the time between birth and age at tagging remain unknown and the natal origins of a tagged fish cannot be determined. In addition, marine fish release large numbers of propagules that are subject to high rates of mortality during the early stages of life. Therefore, the likelihood that a tagged larval or juvenile fish will be recaptured is low, requiring the application of unfeasibly large numbers of tags to ensure even a few recaptures. The likelihood of recapture is further diminished when tagged fishes travel great distances and have large population sizes. The combined limitations of artificial tagging studies have prompted growing interest in the use of natural tags to elucidate movements of migratory animals and fishes in particular (Hobson 1999; Kennedy et al. 2002; Rubenstein and Hobson 2004; Gillanders 2005a, 2005b; Herzka 2005).

Two central questions in the study of migratory behavior are where fish migrate during their time at sea and whether individual fish return to spawn in the same location in which they were born (referred to as natal homing). However, the oceanic movements

of nearly all migratory fishes are so poorly understood that this phase of their life cycle is often regarded as a black box in descriptions or models of spatial population dynamics (Metcalf et al. 2002; McDowall 2003). An area of particular interest is the degree to which populations mix in the marine environment. For species that travel large geographic distances over several years, the potential for significant mixing of populations is very high. However, mixing estimates are generally unknown for most species, further obscuring the processes that shape movement patterns.

Despite the potential for mixing, some marine and anadromous species are known to have remarkably high rates of return to their natal location to spawn, a phenomenon known as philopatry. For example, the overwhelming majority of sockeye salmon are philopatric, with as little as 0.1% to 1.0% straying rates to other spawning habitats (Quinn et al. 1999). Natal homing may be less precise but still significant in other marine fishes, such as the weakfish (*Cynoscion regalis*) which exhibits up to 81% spawning site fidelity (Thorrold et al. 2001). Philopatry in migratory species maintains relatively isolated breeding populations or stocks from a specific spawning location (Begg et al. 1999). Exchange of breeding individuals among populations could influence genetic drift, the development of divergent characters, and local adaptations (Futuyma 1998; Roughgarden 1998; Conover et al. 2006). Stock-specific population dynamics are similarly affected by migratory behavior and natal homing, with the degree of population self-replenishment dependent on philopatry and straying rates (Webster et al. 2002).

In order to fully characterize the spatial population dynamics of species and their individual stocks, the degree of population mixing in the marine environment and rates of philopatry must be quantified. Answering such questions depends on the ability to

accurately assign fish to their natal locations. Natural tags allow natal classification and are thus promising tools to answer these questions about the spatial population dynamics of migratory fishes.

1.2 STUDY SPECIES

American shad *Alosa sapidissima* (Wilson) are anadromous alosine clupeids occupying coastal habitats from the St. Johns River in Florida to the St. Lawrence River in Quebec (Limburg et al. 2003). American shad mature after 3 to 7 years of migration in the marine environment, after which they return to spawn in fresh water (Maki et al. 2001). Mark-recapture studies were conducted on adult American shad for several decades to determine the spatial distribution of individual stocks and oceanic migration rates. This tagging effort showed that American shad undertook long-distance migrations along the Atlantic coast and stocks appeared to follow similar routes along the way, segregating into different geographic areas only in the winter (Leggett 1977; Dadswell et al. 1987).

The general movements of adult American shad at sea appear to be predictable and tuned to the changing seasons. While overwintering, American shad form three discrete offshore aggregations off Florida, the Middle Atlantic Bight, and the Scotian Shelf (Dadswell et al. 1987). After overwintering, American shad follow seasonally shifting isotherms, preferentially traveling in waters with bottom temperatures between 3° and 15°C (Neves and Depres 1979). The thermal band shifts northward from spring to summer, with mature American shad assorting into their spawning rivers as the band arrives at the appropriate latitude. Upriver migration appears highly dependent on river

temperature as well, with peak upriver spawning migrations, or runs, occurring when water temperatures are between 15°-20°C (Leggett and Whitney 1972; Quinn and Adams 1996). As a result, the earliest runs begin in December in Florida and conclude in July in Quebec (Limburg et al. 2003). Yet these migratory routes are not rigid pathways. Dadswell et al. (1987) show that chance apparently plays a role in determining which tidal basins fish enter first. American shad may not be strictly bound by isotherms either, with individuals passing outside “preferred” ranges (Melvin et al. 1986; Dadswell et al. 1987). Once the spawning season is over, post-spawning and non-spawning adults spend the summer in the Bay of Fundy, and tagging studies found representatives from southern and northern stocks in these feeding aggregations (Talbot and Sykes 1958; Dadswell et al. 1987). The migratory cycle begins again as northern waters cool in the fall and American shad head southward and offshore to their overwintering grounds.

Several lines of evidence suggest that American shad home to their natal stream with some degree of precision. Early tagging studies in Albermarle Sound, North Carolina (Hollis 1948) and the York River, Virginia (Nichols 1960b) indicated a homing tendency, but these studies relied on a very small number of returns (3 and 19 fish, respectively). A more substantial tagging study on 1981 and 1982 spawning runs reported 97% spawning fidelity to the Annapolis River, Nova Scotia (Melvin et al. 1986). However, this study involved tagged adults and thus was only able to assess fidelity to a river of previous spawning, with the assumption that the spawning river was their natal one (Melvin et al. 1986). More recently, artificially-induced marks in otoliths of hatchery-reared larval American shad allowed direct estimates of straying rates among several Chesapeake Bay river systems. Using these hatchery marks, Olney et al (2003)

reported only 4% of returning spawners in the James River originated from other rivers, while McBride et al. (2005) estimated negligible numbers of strays from the Susquehanna River to the Delaware River. Meristic characters such as fin ray counts and morphometric characters such as fork length showed significant differences in mean values between fish from different geographic regions, rivers and tributaries, suggesting philopatry and divergence in these characters on a fine spatial scale (Carscadden and Leggett 1975b; Melvin et al. 1992). In addition to phenotypic differences, some genetic divergence has been observed among American shad stocks. Mitochondrial DNA (mtDNA) and microsatellite DNA polymorphisms were subtly different between stocks (Nolan et al. 1991; Waters et al. 2000). Neither Waters et al. (2000) nor Nolan et al. (1991) detected fixed alleles for individual stocks, but relied on stock-specific frequencies of genotypes. The lack of strong genetic differentiation between stocks does not invalidate a hypothesis of significant natal homing rates, since as little as 1% straying between subpopulations can maintain genetic homogeneity (Lewontin 1974). The small differentiation observed by Nolan et al. (1991) therefore suggests significant philopatry. However, because of this sensitivity to low exchange rates of individuals among populations, genetic analyses can only determine whether there is either some unknown yet significant degree of straying or negligible straying, and cannot quantify actual rates of philopatry.

Estimates of natal homing rates and connectivity among American shad stocks are important for many aspects of American shad biology. The extent of divergence in phenotypes and genotypes in some part depends on how reproductively isolated stocks are from one another. Philopatry has been invoked in explanations for small-scale

divergence in anatomical characters as well as large-scale differences in life histories such as latitudinal variation in repeat spawning behavior (Leggett and Carscadden 1978; Melvin et al. 1992). If American shad do not home with some degree of precision, new explanations for observed differences between stocks will be required. Finally, natal homing must be understood in order to develop appropriate management strategies for exploited American shad stocks. If American shad stray significantly, then depleted stocks may experience a “rescue effect” from other abundant stocks. Conversely, high rates of philopatry would suggest that each stock must be managed individually with careful regard for stock-specific characters. Assessments of natal homing rates will thus inform investigations into American shad biology as well as fisheries management plans.

Much attention has been paid to the extensive anthropogenic harvests of American shad over the years. American shad have been utilized as a food source since before European settlement of North America, and commercial exploitation began in earnest during the 19th century (Limburg et al. 2003). American shad fisheries historically harvested spawning adults during their upriver migrations. These fisheries were stock-specific and directed at individuals in the upper reaches of their freshwater habitats. Economic pressures and technological advances at the end of the 1800s allowed increased harvesting rates and extraction in estuarine habitats (Limburg et al. 2003). Harvests peaked with approximately 23 thousand metric tons landed at the turn of the century (ASMFC 1999). Despite efforts to supplement stocks with hatchery-reared larvae, populations declined precipitously; only 680 metric tons were landed in 1993 (ASMFC 1999). Offshore coastal ocean intercept fisheries developed in the 1980s and accounted for 45% of total landings in 2001 (ASMFC 2002). Continued downward

trends in stock abundances indicate that the American shad fishery is fully exploited (Kocik 1998). These trends led to the Atlantic States Marine Fisheries Council to close the ocean intercept fishery at the end of 2004, and moratoria on in-river fisheries exist for some rivers including the James, York, and Rappahannock (Olney and Hoenig 2001). Although these management steps were taken to reduce harvest mortality of American shad, pressures to reopen in-river and coastal fisheries persist and a mixed-stock fishery still exists in Delaware Bay.

To develop effective management strategies, the stock composition of harvests taken by coastal intercept fisheries must be known. Although American shad have been extirpated from many rivers throughout their range and historic populations inhabited nearly 140 river systems, at least 68 discrete spawning populations persist (Limburg et al. 2003). Tag-return data suggest that offshore aggregations of American shad may include individuals from many spawning stocks across their native range (Dadswell et al. 1987). Offshore harvests could therefore contain fish from multiple stocks at a given sampling location. However, certain stocks are more depleted than others (Limburg et al. 2003) and may be adversely and differentially affected by mixed-stock fisheries. Moreover, because American shad travel significant distances, a geographically restricted fishery may in fact exert mortality pressure on stocks originating thousands of kilometers away. Management strategies that ensure sustainable harvests of all stocks require detailed knowledge of the relative contributions of spawning populations to these mixed-stock harvests.

Assessments of stock-specific variation in American shad DNA sequences allow investigators to conduct mixed-stock analyses (MSAs) on offshore landings. The direct

application of genetic techniques to MSAs was illustrated by Brown et al. (1999), who investigated the origin of American shad collected by ocean intercept fisheries off Virginia and Maryland by comparing mtDNA restriction fragment patterns. Brown et al. (1999) used maximum-likelihood estimation to determine relative contributions of spawning stocks to the harvested samples and found that samples were comprised of several stocks. The proportional abundances of stocks in harvested samples varied temporally and geographically, suggesting that management strategies could not rely on data from a single year or location and continual monitoring of harvest composition may be required (Brown et al. 1999). Although maximum-likelihood estimators based on mtDNA and microsatellites show some promise for MSA on American shad, these techniques are still in development (Nolan et al. 2003). MSA traditionally relies on stock-specific genetic divergence to determine relative stock contributions by maximum likelihood estimation (Pella and Milner 1987; Utter and Ryman 1993). However, the utility of this approach is limited in species with moderate genetic divergence and numerous source populations (Smouse et al. 1990), such as American shad. In addition, stock identifications based on genetic analyses are significantly complicated by the inclusion of fish that might have originated from hatcheries. Naturally occurring non-genetic markers that allow natal origins to be determined with minimal classification error would be useful in assessing compositions of American shad assemblages in the marine environment.

Migratory patterns and mixed-stock compositions of American shad in the marine environment have to date been described for adult fish only. Knowledge on the behaviors and distributions of immature fish after exiting fresh water is lacking, owing

principally to the difficulties associated with tagging small fish. As a result, ontogenetic variations in marine migrations are unknown and American shad are assumed to follow similar marine pathways at all stages of their life history. This is undoubtedly an incorrect assumption, given that American shad exhibit size-related variability in their fresh water emigration timing (Limburg 1996a) and are likely to undergo further ontogenetic niche shifts after they enter the marine environment. Size and age also appear to influence distributions and migration distances in other species such as Atlantic herring (Ruzzante et al. 2006), Pacific sardine (Smith 2005), American eel (Helfman et al. 1987), striped bass (Secor and Piccoli 1996), brook charr (Lenormand et al. 2004), and capelin (Fauchald et al. 2006). An otolith natural tag approach offers a unique opportunity to estimate mixed-stock compositions of immature American shad in the marine environment and thereby aid our understanding of stage-specific migratory behaviors for this species.

1.3 OTOLITH CHEMISTRY

Fish ear bones, or otoliths, have proved to be a valuable tool for discovering natal origins of individuals and determining connectivity rates between subpopulations of coastal marine species. Otoliths are calcareous structures in the inner ears of teleost fishes that aid in hearing and balance (reviewed by Popper and Lu 2000). Several properties of otoliths make them useful recorders of life history events. First, otoliths grow by the continuous accretion of calcium carbonate layers throughout the life of the fish. When otoliths are sectioned these layers appear as daily rings in early life and year rings subsequently that can be counted to determine the age of the fish (Campana and

Nielson 1985). Second, once otolith material accretes, it is inert and is not metabolically reworked. The chemical composition of a layer therefore remains stable over time (Campana 1999). Third, the chemical composition of a layer reflects, to some degree, the composition of the ambient water in which the fish resides at the time of accretion (Bath et al. 2000). Finally, otolith material derives mainly from the ambient water with only a minimal amount contributed by diet (Farrell and Campana 1996; Gallahar and Kingsford 1996; Walther and Thorrold 2006). Taken together, these properties mean that the environmental history of a fish can be reconstructed by sampling the section of the otolith corresponding to the life history stage of interest (Campana and Thorrold 2001). The development of precise measurements of otolith composition using inductively coupled plasma mass spectrometry (ICP-MS) and isotope ratio mass spectrometry (IR-MS) has made such restructurings possible (Thresher 1999; Kennedy et al. 2000; Barnett-Johnson et al. 2005).

There is good evidence to suggest that natural stable isotope markers vary geographically in a way that produces distinguishable local signatures in the environment. Pronounced latitudinal gradients in the isotopic ratios $\delta^{18}\text{O}$ and δD exist primarily due to the preferential retention of ^{18}O and D in liquid form and the variation of $\delta^{18}\text{O}$ and δD with mean annual temperature (Bowen 1988). As a result, $\delta^{18}\text{O}$ and δD of local precipitation becomes isotopically lighter poleward, an affect known as the Rayleigh distillation (Dansgaard 1964; Poage and Chamberlain 2001). Because animals incorporate this local groundwater signature into their tissues, $\delta^{18}\text{O}$ and δD have been used to describe latitudinal movements of a variety of terrestrial animals (Schaffner and Swart 1991; Hobson et al. 1999; Rubenstein et al. 2002). Fish incorporate $\delta^{18}\text{O}$ ratios in

their otoliths without metabolic or kinetic fractionation (Thorrold et al. 1997; Høie et al. 2004) and therefore freshwater natal $\delta^{18}\text{O}$ otolith signatures should reliably indicate spawning latitude. Environmental ^{87}Sr : ^{86}Sr ratios are also highly location-specific, although instead of varying along any uniform gradient they reflect the underlying geology of each stream bed (Bricker and Jones 1995; Capo et al. 1998). These geographically distinct ^{87}Sr : ^{86}Sr ratios are reliable markers recorded in otoliths and have proved useful in determining natal origins of salmonids (Kennedy et al. 1997; Kennedy et al. 2000). Finally, trace elemental compositions, expressed as ratios to calcium, recorded in otoliths have similarly allowed separation of fish according to the river in which they were born (Thorrold et al. 1998b). Together, these elemental abundances and isotope ratios allow relatively fine discrimination of natal signatures recorded in otoliths and are useful markers when classifying fish of unknown origins.

In practice, otolith chemistry has been used to determine the natal origins of highly migratory marine species and estimate rates of natal homing of spawning fish. An assay of the central portion of an adult otolith provides a signature indicative of the location in which the fish spent the early part of its life (Campana and Thorrold 2001). Habitat-specific otolith signatures are quantified by collecting and analyzing otoliths from juveniles that have not yet left their nursery habitats (Thorrold et al. 1998a). Fish of unknown origins from the same year-class are then classified according to their natal location by comparing signatures from the central portion of their otoliths to the ground-truthed juvenile signatures. If the degree of inter-annual variability in natal signatures is unknown, each cohort must be ground-truthed and adults should only be compared to the database describing the cohort to which they belong (Swearer et al. 2003). The

classification of returning adults based on natal origin can be highly accurate if the ground-truthed juvenile signatures show distinct separation in multivariate space. This method allowed Thorrold et al. (2001) to assess rates of natal homing in weakfish (*Cynoscion regalis*), a species previously thought to have a panmictic population structure along the Atlantic coast. Despite genetic evidence suggesting no spatial population structure (Cordes and Graves 2003), 60-81% of weakfish return to spawn in their natal estuary (Thorrold et al. 2001).

Otoliths are ideal tools for use in investigations of spatial population dynamics of American shad. Previous work has demonstrated that American shad record distinguishable natal signatures in their otoliths. Thorrold et al. (1998b) collected juvenile otoliths from the Connecticut, Hudson and Delaware rivers in 1994. The relative abundances of elements including Sr, Ba, Mg and Mn were quantified using isotope dilution ICP-MS. These four elements differed significantly among rivers and discriminant function analyses (DFAs) assigned fish to their natal river with approximately 90% accuracy. These results indicate that American shad record unique signatures from their freshwater habitats that are readily distinguishable using mass spectrometric methods. In order to accurately estimate mixed-stock compositions and natal origins of spawners, a ground-truthed database of river-specific signatures must be include as many source rivers as possible to avoid estimation biases (Fabrizio 2005). In addition, subsequent collections will allow signatures to be compared between years to assess inter-annual variability in the composition of otoliths from a particular river. These data will allow adults to be classified to most potential source rivers and lay the groundwork for investigations of migratory dynamics.

Information on mixed-stock compositions and natal homing would be useful not only to biologists interested in a more complete picture of American shad, but also to fisheries managers charged with the protection and conservation of depleted stocks. Though genetic data have been used for MSAs with some success, the inclusion of otolith chemical analyses could supplement and enhance estimates of the composition of offshore harvests. Understanding where stocks migrate and the degree of mixing during migrations are critical for the development of sound management strategies that protect the most significantly depleted stocks. Confident assessments of natal homing will be necessary to determine the resiliency of stocks under intense harvest pressure. This thesis aims to address these issues for American shad with the goal of informing effective management strategies that ensure the persistence of the species throughout its native range.

1.4 THESIS STRUCTURE

Chapter 2 begins the investigation of geochemical signature variability in juvenile otoliths collected over the course of three years from several rivers. Stable isotope and elemental ratios were quantified using laser ablation inductively coupled plasma mass spectrometry (ICP-MS) from rivers between Georgia and New Hampshire. Signatures were distinct among rivers and classifications of known-origin juveniles were highly accurate. The chapter also reports on inter-annual variability in juvenile otolith signatures, driven primarily by $\delta^{18}\text{O}$ values. The ground-truthed juvenile otolith database is then drawn upon to identify natal origins of spawning adults in the York River system (Virginia). The results suggest that while most American shad home to their natal rivers,

discrimination among tributaries within a river is less precise. These results imply that, the population in the Mattaponi River may act as a source that subsidizes the Pamunkey River population.

Chapter 3 expands the juvenile database to include all major spawning rivers throughout the native range of American shad from Florida to Quebec. This database draws upon analyses of juvenile otoliths and water samples from 20 rivers in 2004, covering approximately 2700 km of coastline and 19 degrees of latitude. The relationship between water and otolith composition in 5 rivers where both were collected allowed otolith composition to be predicted for those rivers where only water was sampled for some, but not all, geochemical signatures. Classification accuracies based on these actual and predicted otolith signatures remained high, allowing reliable estimates of migrant natal origins.

Chapter 4 uses the large ground-truthed database from 2004 to estimate mixed stock compositions of one-year-old fish collected during their marine migrations. Fish were collected along the coast of Maine in the spring of 2005 and Minas Basin in the summer of 2005. This analysis allowed the assessment of both geographical and seasonal variation in composition. Mixed-stock compositions appeared to differ significantly from those previously reported for tagged adult migrants, indicating the complexity of American shad migrations in the marine environment and suggesting ontogenetic shifts in distributions. The thesis concludes in Chapter 5 by placing these findings in a theoretical context and suggesting future work. An appendix describes an experimental approach to determine the relative contributions of food and water to otolith material, a key assumption in all studies employing otoliths as natural tags.

Chapter 2

GEOCHEMICAL SIGNATURES IN OTOLITHS RECORD NATAL ORIGINS OF AMERICAN SHAD

ABSTRACT

The extent to which populations of migrating anadromous fishes exchange individuals influences life history dynamics and local population persistence. Geochemical signatures in otoliths of American shad (*Alosa sapidissima*) were used to determine natal origins and estimate rates of straying among river-specific populations along the Atlantic coast of the United States. Stable isotope ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $^{87}\text{Sr}:^{86}\text{Sr}$) and elemental (Mg:Ca, Mn:Ca, Sr:Ca and Ba:Ca) signatures in otoliths of juvenile American shad from rivers from Georgia to New Hampshire varied significantly, allowing for an average of 91% cross-validated accuracy when classifying individual fish to their natal rivers. There was significant inter-annual variability in geochemical signatures from several rivers, due largely to differences in $\delta^{18}\text{O}$ values among years. The ground-truthed geochemical signatures in otoliths of juvenile American shad were used to identify natal origins of spawning adults in the York River system (Virginia). Approximately 6% of the adults were strays from other rivers. Of the remaining adults, 79% were spawned in the Mattaponi River and 21% were spawned in the Pamunkey River. The results suggested that while most American shad spawning in the York River were homing to their natal river there was much less fidelity to individual tributaries. Small-scale straying allowed fish spawned in the Mattaponi River to subsidize spawning in the Pamunkey River, which has experienced persistent recruitment failure.

2.1 INTRODUCTION

Anadromous fishes often display complicated migration patterns that present challenges to investigators seeking to understand the relationship between movements, life history traits and population dynamics. Outstanding questions include determining the origins of migrating fish, the degree of homing to natal rivers and the effects of fishing pressure directed at small, tributary-specific stock components. While significant work has gone into addressing these questions, direct tests of hypotheses concerning natal origin and migratory behavior are difficult with traditional tagging techniques (Dingle 1996; Thorrold et al. 2002). Most information on anadromous migrations comes from mark-recapture studies that apply a tag to a fish and attempt to reconstruct a route once that tag is recovered (Dadswell et al. 1987; Hendry et al. 2004). While the tags employed are becoming increasingly sophisticated (e.g., Block et al. 2005), this approach can only yield information about movements subsequent to tag application after the fish reaches some minimum size (Webster et al. 2002). As a result, traditional tags are unable to provide data about early life history movements and spawning origins of fishes, both of which are crucial aspects of population dynamics (Metcalf et al. 2002).

The use of natural geochemical tags in animal tissues provides an alternative marking technique in species that are difficult to tag using conventional approaches (Rubenstein and Hobson 2004). Recently, fish otoliths have been shown to be particularly useful natural tags (e.g., Thorrold et al. 2001). Otoliths are paired calcareous structures in the inner ear of fishes that are formed by the sequential addition of stable and inert layers of carbonate from birth (Campana and Nielson 1985; Campana 1999). The composition of otolith aragonite reflects, at least to some degree, the chemistry of

ambient waters at the time of deposition (Bath et al. 2000; Walther and Thorrold 2006). Thus, otoliths from fish spawned in chemically distinct waters will record unique signatures reflective of those habitats and continue to record movements between distinct waters over their lifetimes.

American shad (*Alosa sapidissima*) is an excellent candidate species to apply analyses of otolith geochemistry as there is a pressing need to understand the migratory dynamics of American shad. Most populations along the Atlantic coast are fully exploited or under moratorium (Olney and Hoenig 2001) and all are at a fraction of their historic abundances (Limburg et al. 2003). Anadromous alosine clupeids native to the east coast of North America, American shad spawn in fresh water habitats from the St. Johns River in Florida to the St. Lawrence River in Quebec (Limburg et al. 2003). After developing in fresh water, juveniles migrate to the coastal ocean where they spend 3 to 7 years before returning to spawn in fresh water upon reaching maturity (Maki et al. 2001; Collette and Klein-MacPhee 2002). While adult American shad are presumed to return to their natal river to spawn, this hypothesis has only been tested using traditional tagging and genetic approaches (Melvin et al. 1986; Nolan et al. 1991; Waters et al. 2000), methods that are often unable to identify natal origins.

Previous work using otolith chemistry has shown that elemental signatures in juvenile American shad from three rivers were highly distinct (Thorrold et al. 1998b). This chapter expands on these studies by examined geochemical signatures in juvenile American shad otoliths from 12 rivers throughout their native range, including juveniles from the same river over multiple years. Juvenile signatures were then used to estimate

natal origins of adults spawning in the York River system to determine homing on both a river and tributary scale.

2.2 MATERIALS AND METHODS

2.2.1 Sample collections

Juvenile American shad were collected from 12 rivers along the Atlantic coast of the United States in 2000, 2001 and 2002 (Table 2.1). Samples were obtained from three of these rivers over consecutive years: in the Hudson River in 2000 and 2001 and in the Mattaponi and Pamunkey Rivers in 2000, 2001 and 2002. The Mattaponi and Pamunkey Rivers are the two tributaries that join to form the York River at Westpoint, Virginia. The remaining nine rivers were sampled for one year only. Juveniles were collected while residing in fresh water or the upper estuarine environment prior to migration to marine habitats to ensure individuals were collected from their natal river. Collections occurred each year in summer, fall and winter months depending on spawning latitude and were timed to coincide with high juvenile abundance in each river following spawning migrations. Push nets and beach seines were used to obtain representative samples and specimens were subsequently returned to the lab and frozen whole. An average of 25 juveniles (range: 18-29) from each river and in each year were included in the analyses (Table 2.1).

Adult American shad were collected during their upriver spawning migration in 2002 in staked gill nets located in the middle reaches of the York River, approximately

Table 2.1 Juvenile American shad collected for analyses of otolith chemistry to ground-truth signatures in each spawning habitat. Fork lengths (mean \pm standard deviation) are reported for all rivers, except for the Santee-Cooper where lengths were unavailable. Spawning latitude is the location of the highest accessible spawning habitat within each river.

River	Spawning latitude (decimal degrees)	<i>n</i>	Year Collected	Fork length (mm)
Exeter	42.97	28	2001	92 \pm 8
Connecticut	42.58	28	2001	72 \pm 7
Hudson	42.65	27	2000	52 \pm 8
Hudson	42.65	28	2001	60 \pm 4
Delaware	41.97	21	2000	56 \pm 14
Susquehanna	40.25	20	2000	141 \pm 9
Upper Chesapeake	39.66	29	2000	71 \pm 4
Potomac	39.00	23	2000	70 \pm 3
Rappahannock	38.31	21	2000	70 \pm 13
Mattaponi	37.78	27	2000	53 \pm 5
Mattaponi	37.78	28	2001	57 \pm 6
Mattaponi	37.78	24	2002	54 \pm 5
Pamunkey	37.68	18	2000	51 \pm 8
Pamunkey	37.68	29	2001	58 \pm 6
Pamunkey	37.68	19	2002	48 \pm 10
Santee-Cooper	34.24	26	2000	-
Altamaha	33.09	24	2000	63 \pm 12

24 km below the confluence of the Mattaponi and Pamunkey tributaries (Figure 2.1). This region of the York River historically supported important gill net fisheries and is the likely site of future exploitation if the current ban on fishing is lifted (Olney and Hoenig 2001). Scales from a mid-lateral location on the left side posterior to the pectoral-fin base were removed from each adult and retained dry in paper envelopes for estimating age. Sagittal otoliths were removed and stored in numbered tissue culture trays for subsequent chemical analyses. A total of 78 adults were included in the analyses. This total was a subsample (78 of 384 female fish) of randomly selected individuals in proportion to the total catch in each week of fishing.

2.2.2 Otolith and scale preparation

Frozen fish were thawed, measured (fork length \pm 1 mm) and dissected to remove sagittal otolith pairs. Once removed, otoliths were rinsed in distilled water, dried and mounted on petrographic glass slides with cyanoacrylic glue. One otolith of each pair was ground to the sagittal midplane using 30 and 3 μ m lapping film for elemental and Sr isotope analyses. Once ground, the otolith was sonicated for 2 minutes in ultrapure water, triple-rinsed with ultrapure water and air-dried under a laminar flow hood for 12-24 h. All cleaning took place in a class 100 clean room. The second otolith of the same pair was ground to just above the midplane to leave the required amount of otolith material for C and O isotope analyses. Adult otoliths were mounted and ground to the midplane using similar methods. Otoliths were then sonicated, triple-rinsed and dried in a class 100 clean room. Adult scales were cleaned with a dilute bleach solution, mounted

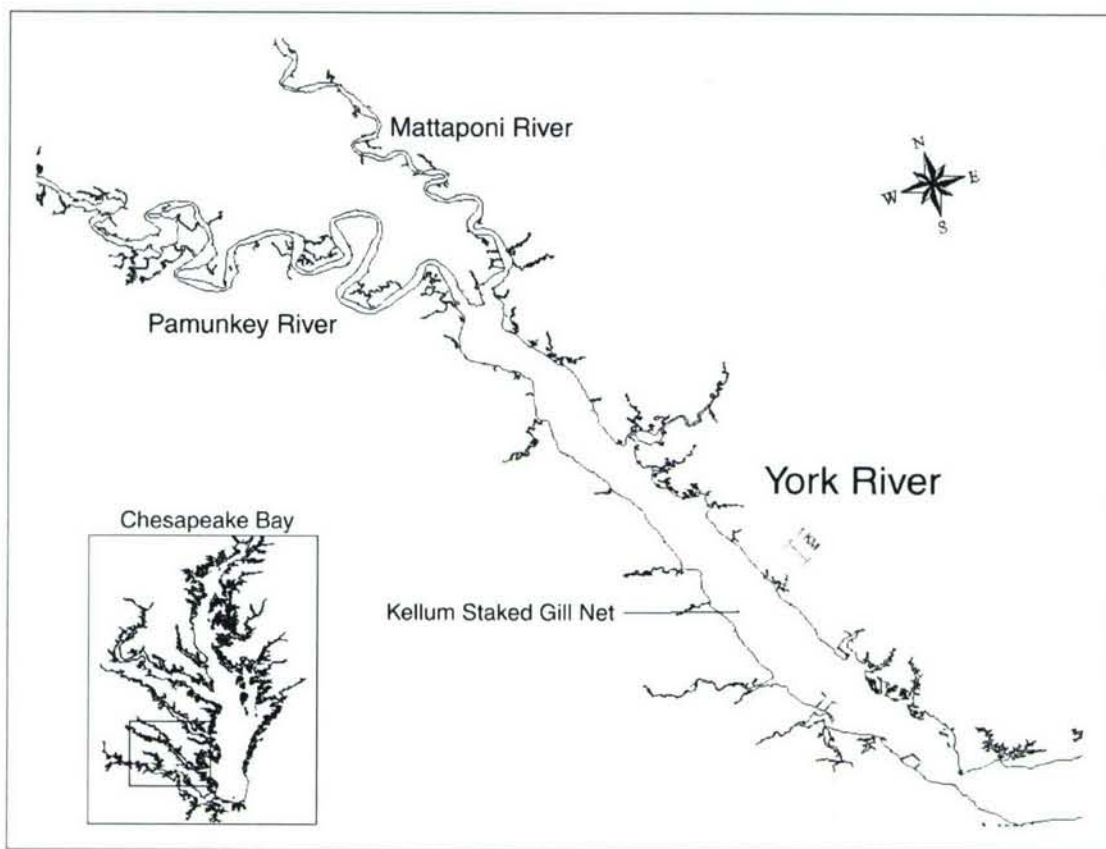


Figure 2.1 York River system in Virginia, USA, indicating the location of spawning adult collections at the Kellum staked gill net downstream of the confluence of the Mattaponi and Pamunkey Rivers.

and pressed on acetate sheets and read on a microfilm projector to estimate ages following the methods of Cating (1953).

2.2.3 Geochemical analyses

2.2.3.1 *Laser ablation ICP-MS*

Juvenile otolith pairs were analyzed for a suite of trace elemental and isotopic ratios to produce a combined river-specific signature. The first otolith of each pair was analyzed with inductively coupled plasma mass spectrometry (ICP-MS) on a Thermo Finnigan Element2 single collector ICP-MS coupled to a New Wave Research UP213nm Nd:YAG laser ablation system. The laser software was used to trace a 200 x 200 μm ablation raster centered on the nucleus and extending toward the posterior lobe of each otolith. Ablated material was carried by a He gas stream from the laser cell to the ICP-MS where it was mixed with Ar sample gas and a wet aerosol (2% HNO_3) supplied by a self-aspirating (20 mL min^{-1}) PFA nebulizer in the concentric region of the quartz dual inlet spray chamber.

Elemental ratios of Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca were quantified in the juvenile otoliths by monitoring ^{25}Mg , ^{48}Ca , ^{55}Mn , ^{86}Sr and ^{138}Ba in ablated material. Instrument blanks (2% HNO_3) and standards were analyzed at the beginning, middle and end of each block of 10 otoliths. A blank value was calculated for each sample by linearly interpolating between measured blanks. Those blank values were then subtracted from raw measured elemental ratios to remove background intensities from measured

counts. A dissolved otolith certified reference material (CRM - Yoshinaga 2000), diluted to a Ca concentration of 40 g.g⁻¹, was used to correct for instrument mass bias following the approach of Rosenthal et al. (1999). This approach measures elemental ratios in the matrix-matched CRM to quantify mass bias. The mass bias correction factor ($C_{Me,Ca}$) between an element (Me) and Ca was quantified by comparing the measured elemental ratio in the CRM ($M_{Me/Ca}$) to the known elemental composition of the CRM ($S_{Me/Ca}$) as follows:

$$(2.1) \quad C_{Me/Ca} = \frac{S_{Me/Ca}}{M_{Me/Ca}}$$

The CRM was measured every 5 samples, and the correction factor was linearly interpolated between measurements to produce a correction factor for each measured otolith sample. This mass bias correction factor is then multiplied by the blank-corrected otolith Me/C value to obtain the true Me/Ca value. Measurement precision was assessed by running a 40 g.g⁻¹ solution of an internal laboratory standard consisting of powdered otoliths. External precision (relative standard deviations) for the lab standard ($n = 92$) were as follows: Mg:Ca = 12%, Mn:Ca = 3%, Sr:Ca: 0.3%, and Ba:Ca: 0.6%).

Strontium isotope ratios ($^{87}\text{Sr}:^{86}\text{Sr}$) were analyzed in the same otolith used for elemental ratio measurements. Otoliths were assayed using a Thermo Finnigan Neptune multiple collector ICP-MS coupled to a 213nm laser ablation system. The laser software was used to trace out a 250 x 200 μm raster centered on the nucleus, extending toward the posterior lobe of each otolith and adjacent to the raster ablated for elemental ratio measurements. Typical raster placement is depicted in Figure 2.2. Ablated material was carried by a He gas stream from the laser cell to the ICP-MS where it was mixed with an Ar sample gas and a wet aerosol in a spray chamber as described above for the elemental

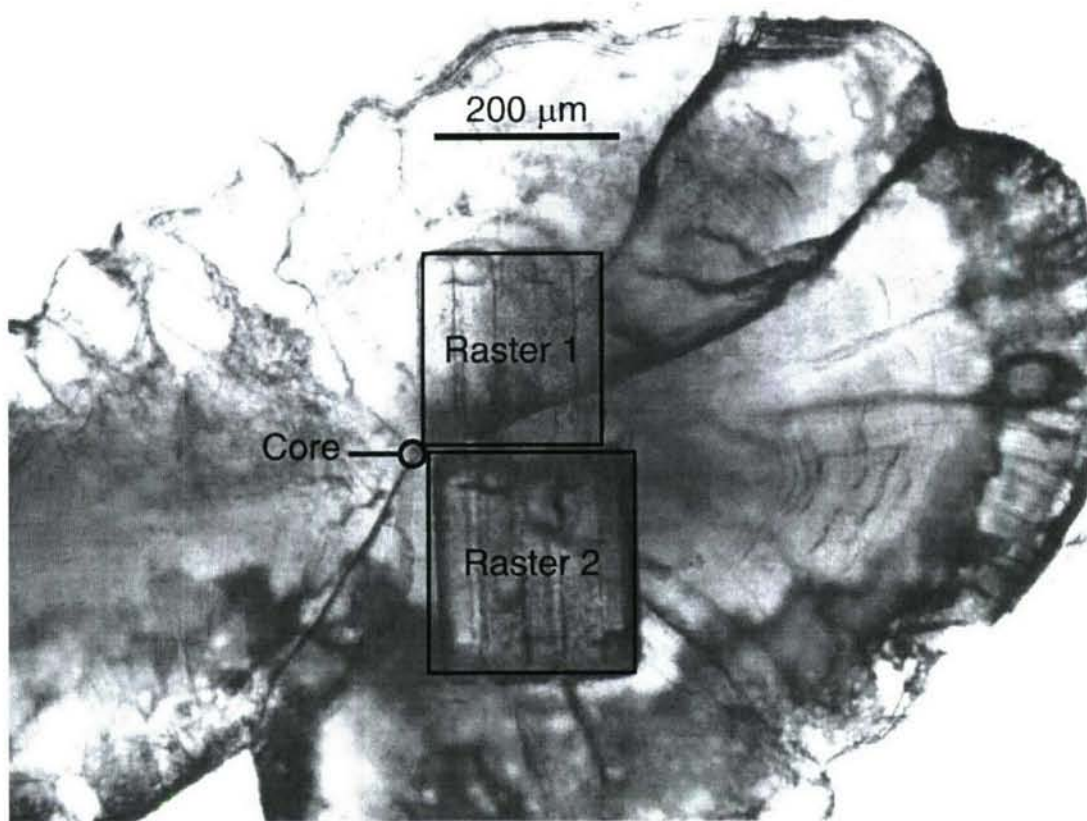


Figure 2.2 Photo of typical juvenile otolith after ablation on the single collector ICP-MS (Raster 1) and the multiple collector ICP-MS (Raster 2). Both rasters were placed adjacent to the core on the posterior lobe of the otolith.

analyses. The core regions of adult American shad otoliths were ablated and analyzed for Sr:Ca and $^{87}\text{Sr}:^{86}\text{Sr}$ ratios simultaneously on the multiple collector ICP-MS.

Although there are a number of potential interferences on Sr isotopes in carbonates, including Ca dimers, Ca argides and doubly-charged Er and Yb (Woodhead et al. 2005), only Rb and Kr isotopes present significant difficulties for accurate and precise analyses of $^{87}\text{Sr}:^{86}\text{Sr}$ in otoliths (Barnett-Johnson et al. 2005; Jackson and Hart 2006). The correction method followed the strategy outlined by Jackson and Hart (2006) to remove Kr interferences on ^{86}Sr . Briefly, Kr was subtracted from the mass 84 intensity until the $^{84}\text{Sr}:^{88}\text{Sr}$ value equaled the natural abundance ratio of the isotopes (0.006755). The resulting Kr value was then used to account for the ^{86}Kr contribution on ^{86}Sr . A mass bias correction was determined from the measured $^{88}\text{Sr}:^{86}\text{Sr}$ ratios and applied to monitored counts of ^{85}Rb to remove the contribution of ^{87}Rb on ^{87}Sr intensities. This procedure obtains the mass-bias corrected sample value $^{87}\text{Sr}:^{86}\text{Sr}_{\text{true}}$ using an exponential relationship between the measured $^{87}\text{Sr}:^{86}\text{Sr}_{\text{sample}}$, the measured $^{88}\text{Sr}:^{86}\text{Sr}_{\text{sample}}$, and the known value $^{88}\text{Sr}:^{86}\text{Sr}_{\text{certified}}$ where

$$(2.2) \quad ^{87}\text{Sr}:^{86}\text{Sr}_{\text{true}} = \frac{^{87}\text{Sr}:^{86}\text{Sr}_{\text{sample}}}{\left(\frac{^{88}\text{Sr}:^{86}\text{Sr}_{\text{sample}}}{^{88}\text{Sr}:^{86}\text{Sr}_{\text{certified}}} \right)^{\beta}}$$

The exponent β derives from the relationship

$$(2.3) \quad \beta = \frac{\ln\left(\frac{M_{87}}{M_{86}}\right)}{\ln\left(\frac{M_{88}}{M_{86}}\right)}$$

and each M represents the nuclidic mass of the respective Sr isotope. Finally, data were normalized to a SRM987 $^{87}\text{Sr}:^{86}\text{Sr}$ value of 0.71024 based on the mean $^{87}\text{Sr}:^{86}\text{Sr}$ ratio of

SRM987 measured during a given analysis session. The correction strategies produced accurate and precise long-term measurements of liquid and solid standards that were run throughout the otolith analyses. Daily laser sampling of the aragonitic skeleton from a marine sclerosponge ($n = 18$) produced a mean (\pm SD) $^{87}\text{Sr}:^{86}\text{Sr}$ value of 0.70918 (\pm 0.00001), while solutions of SRM987 ($n = 40$) and the otolith CRM ($n = 38$) produced values of 0.71025 (\pm 0.00002) and 0.70915 (\pm 0.00002), respectively. These numbers compare favorably with the global marine $^{87}\text{Sr}:^{86}\text{Sr}$ ratio of 0.70917 (Ingram and Sloan 1992; Woodhead et al. 2005) and the generally accepted $^{87}\text{Sr}:^{86}\text{Sr}$ value of 0.71024 for SRM987 (e.g., Stewart et al. 2001; Aulbach et al. 2004; Jackson and Hart 2006).

2.2.3.3 *Isotope ratio mass spectrometry*

The second otolith from each juvenile was analyzed for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ using isotope ratio mass spectrometry. The core of each otolith was removed using a computer-controlled mill to trace out a 400 x 400 μm raster with a 75 μm drilling depth adjacent to the nucleus and extending toward the posterior lobe. Mean sample mass of the milled otolith powder ($n = 420$) was 43 ± 12 μg (1 SD). Samples were then analyzed on a Thermo Finnigan MAT252 equipped with a Kiel III carbonate device following methods outlined by Ostermann and Curry (2000). Isotopic values were reported relative to Vienna Pee Dee belemnite (VPDB) and expressed in standard δ notation where

$$(2.4) \quad \delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

and R represents the ratio $^{18}\text{O}:^{16}\text{O}$ measured in the sample and standard, respectively.

Long-term precision estimates of the mass spectrometer based on analyses of NBS19 are ± 0.07 for $\delta^{18}\text{O}$ and ± 0.03 for $\delta^{13}\text{C}$ (Ostermann and Curry 2000).

2.2.3.3 Statistical analyses

2.2.3.3.a Juvenile American shad

Laser ablation ICP-MS and IR-MS analyses produced a total of 7 variables for each juvenile: Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, $^{87}\text{Sr}:^{86}\text{Sr}$, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$. All corrected chemical data for each otolith included in the analyses are provided in Appendix 2. Each variable was tested for assumptions of normality and equality of variance-covariance matrices. Normal probability plots, residual analysis, and Box's M-tests indicated that distributions were non-normal and the variance-covariance matrices were not equal. However, because departures from the assumptions were modest and log transformations of the data failed to alter significantly the distributions or the results of the Box's M-tests, raw data were used in all analyses. Geographic differences in multivariate signatures among locations and years were visualized using canonical discriminant analysis (CDA). Canonical variate coefficients provided a useful way to measure the relative importance of each variable to the observed separation among rivers and years. Finally, quadratic discriminant function analysis (DFA) was employed to determine the accuracy with which individual American shad could be assigned to their natal river. A quadratic DFA was used since this procedure does not assume homogeneity of covariance matrices and tolerates modest deviations from normality (McGarigal et al. 2000). The DFA used a

jackknife cross-validation procedure to determine classification accuracy. All statistics were performed using SAS/STAT® software.

2.2.3.3.b Adult American shad

Values of $^{87}\text{Sr}:^{86}\text{Sr}$ and $\text{Sr}:\text{Ca}$ obtained from cores of adult otoliths from the York River in 2002 were compared with $^{87}\text{Sr}:^{86}\text{Sr}$ and $\text{Sr}:\text{Ca}$ signatures from Mattaponi and Pamunkey River juveniles caught in 2000, 2001 and 2002. All corrected chemical data for each otolith included in the analyses are provided in Appendix 2. A maximum likelihood (MLE) estimation program (HISEA) determined the proportion of returning adults hatched in the Mattaponi or Pamunkey Rivers (Millar 1990). Ground-truthed $^{87}\text{Sr}:^{86}\text{Sr}$ and $\text{Sr}:\text{Ca}$ signatures from Mattaponi and Pamunkey River juveniles were pooled over the 2000, 2001 and 2002 year classes to parameterize the MLE algorithm. The program calculated variance estimates (standard deviations) on the contribution of each tributary in the adult samples by resampling the mixed stock data 1000 times with replacement.

2.3 RESULTS

2.3.1 Juvenile American shad

There was strong geographical separation of juveniles based on geochemical signatures in otoliths (Figure 2.3). This CDA was performed by combining all juveniles

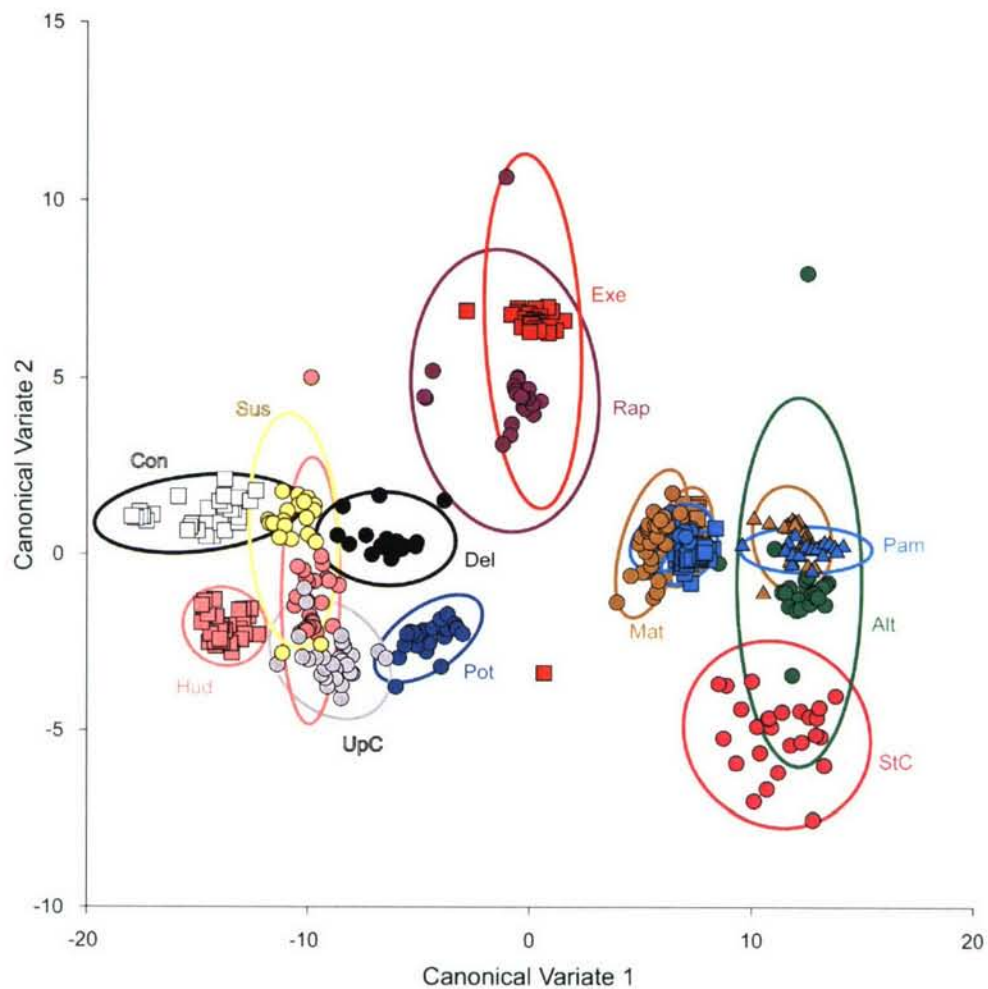


Figure 2.3 Canonical discriminant analysis of juvenile American shad otolith signatures from all fish collected in 2000 (circles), 2001 (squares) and 2002 (triangles) grouped by river of origin and year class. Symbols represent individual fish and ellipses are 95% confidence intervals around each group. River codes are described in Table 2.4.

collected in all rivers and grouping them by both their river of origin and year class. Individuals from different rivers were clearly separated on the first two canonical variates, with the exception of the Mattaponi and Pamunkey tributaries of the York River. However, when the CDA was restricted to these two tributaries the signatures were distinct between locations for a given year (Figure 2.4). Inter-annual variations in signatures were also apparent from the CDAs. Juvenile signatures from the Hudson River were clearly distinct and non-overlapping between 2000 and 2001 (Figure 2.3). Mattaponi and Pamunkey River juvenile signatures occupied similar canonical space in 2000 and 2001 but shifted substantially in 2002 (Figure 2.4).

The magnitude of total canonical structure coefficients reflected the importance of the geochemical variables used to generate the multivariate geochemical signatures (Table 2.2). Oxygen isotopes loaded highly on the first canonical variate, with the latitudinal gradient in $\delta^{18}\text{O}$ accounting for differences among river-specific signatures (Figure 2.5). Separation of signatures along the second canonical variate was primarily driven by variations in $^{87}\text{Sr}/^{86}\text{Sr}$ values. Inter-annual variations in $\delta^{18}\text{O}$ were most responsible for differences in geochemical signatures in the Hudson, Mattaponi and Pamunkey across years on the first canonical variate (Table 2.3). The coefficients in Table 2.3 are not identical to those in Table 2.2 since canonical scores are dimensionless values that describe relationships among the multivariate signatures given the groups that are included in each CDA. Restricting the CDAs to individual rivers over multiple years altered the parameter space and thus the individual canonical structure coefficients, but the relative importance of each ratio in driving inter-annual variability can be determined

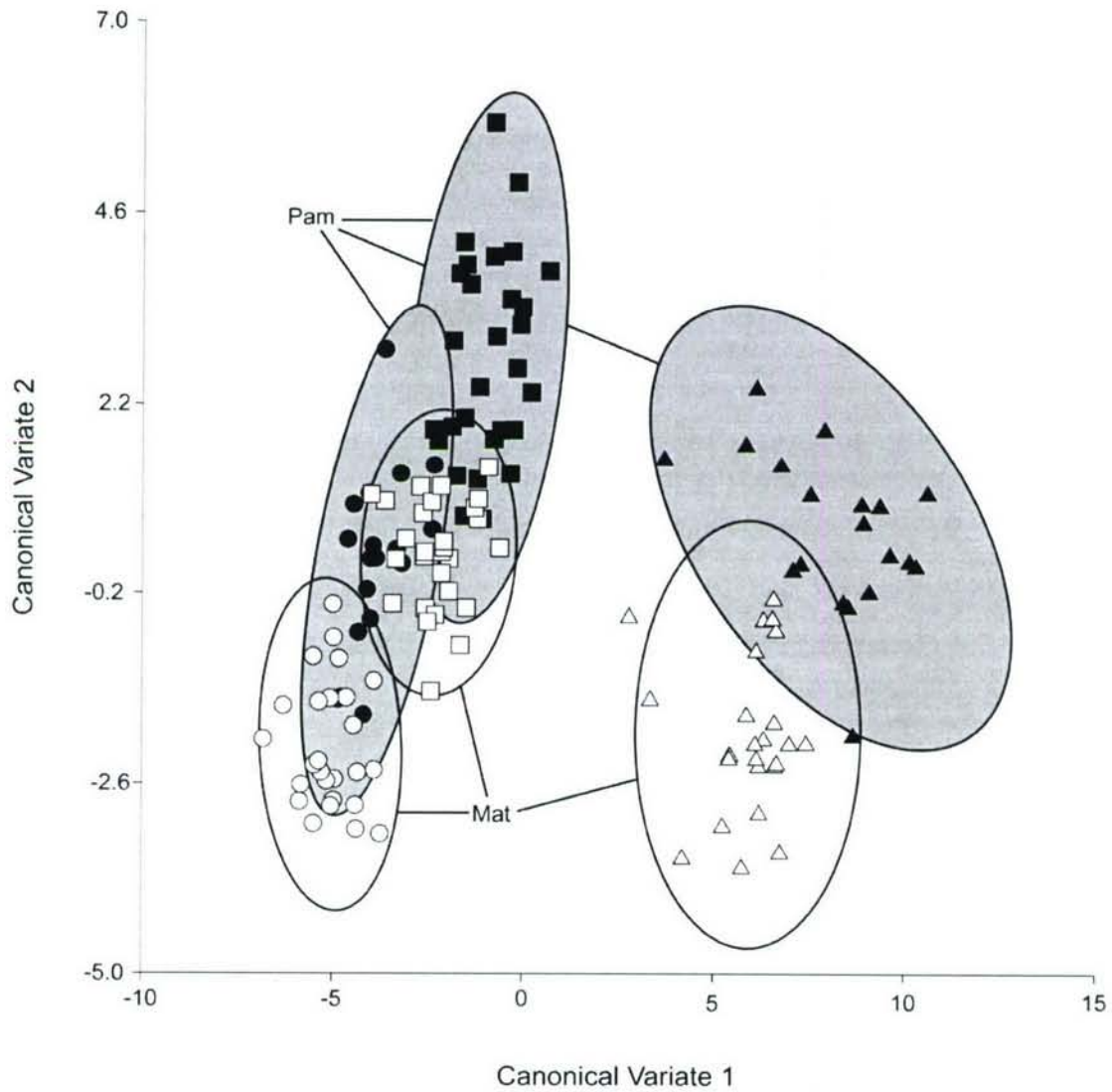


Figure 2.4 Canonical discriminant analysis of juvenile American shad otolith signatures from Mattaponi (filled symbols) and Pamunkey (open symbols) fish collected in 2000 (circles), 2001 (squares) and 2002 (triangles) with 95% confidence ellipses surrounding each group.

Table 2.2 Total canonical structure coefficients for the canonical discriminant analysis performed on juveniles in all rivers across three years. The absolute value of the coefficient indicates the relative importance of the ratio in driving combined signature separation along that variate.

Ratio	Variate 1	Variate 2
$\delta^{18}\text{O}$	0.995	-0.027
$\delta^{13}\text{C}$	-0.730	-0.317
Ba:Ca	0.562	-0.144
Sr:Ca	0.360	-0.442
Mn:Ca	0.275	0.441
Mg:Ca	-0.168	-0.245
$^{87}\text{Sr}:^{86}\text{Sr}$	-0.049	0.974

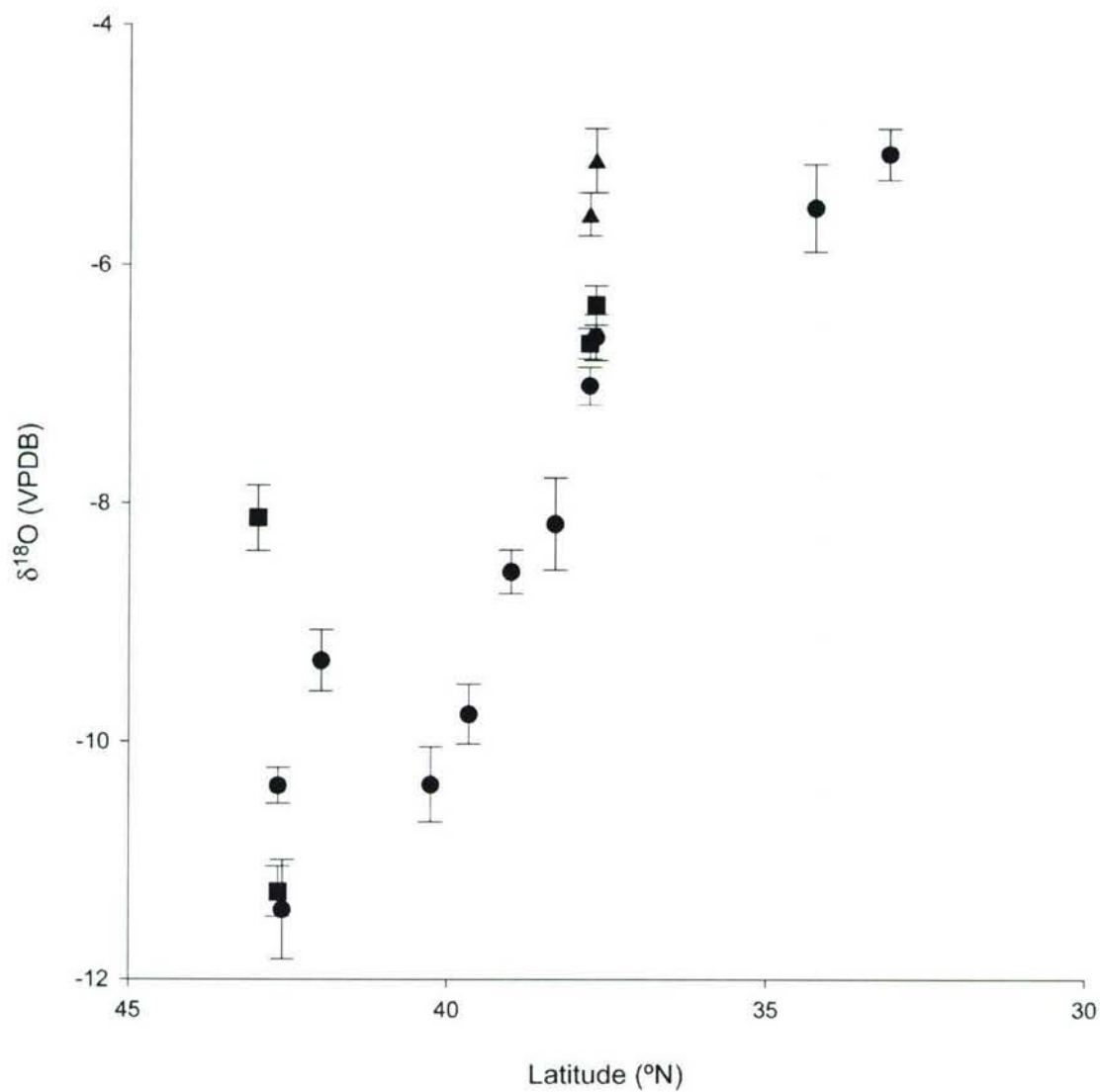


Figure 2.5 Variation of $\delta^{18}\text{O}$ signatures recorded in juvenile American shad otoliths with latitude of spawning habitat from all fish collected in 2000 (circles), 2001 (squares) and 2002 (triangles). Values are means \pm 1 SD of fish grouped by river of origin and year class.

Table 2.3 Total canonical structure coefficients for canonical discriminant analyses of all juveniles in the Hudson (2000 and 2001), Mattaponi and Pamunkey (2000, 2001 and 2002) Rivers. Coefficients are derived from analyses restricted to one river across all collection years to assess the relative importance of each ratio in driving inter-annual variability.

Ratio	Variate 1	Variate 2
Hudson		
$\delta^{18}\text{O}$	0.966	0.120
Ba:Ca	0.576	0.356
$\delta^{13}\text{C}$	-0.506	0.463
Mg:Ca	-0.430	-0.101
$^{87}\text{Sr}:^{86}\text{Sr}$	0.386	-0.463
Mn:Ca	0.205	-0.187
Sr:Ca	-0.241	0.834
Mattaponi		
$\delta^{18}\text{O}$	0.985	-0.014
$\delta^{13}\text{C}$	-0.580	0.126
Mg:Ca	0.519	-0.659
Ba:Ca	-0.135	0.591
Mn:Ca	-0.126	0.678
$^{87}\text{Sr}:^{86}\text{Sr}$	0.098	-0.164
Sr:Ca	0.002	0.921
Pamunkey		
$\delta^{18}\text{O}$	-0.972	-0.051
Ba:Ca	0.743	-0.336
$\delta^{13}\text{C}$	0.634	0.201
$^{87}\text{Sr}:^{86}\text{Sr}$	0.529	-0.231
Mn:Ca	-0.454	-0.669
Sr:Ca	0.404	-0.394
Mg:Ca	-0.012	0.896

by examining the magnitude of the coefficients. The switch in the sign of the $\delta^{18}\text{O}$ coefficient in the two CDAs run separately for the Mattaponi (positive coefficient) and Pamunkey (negative coefficient) rivers is not an indication that they experience opposite trends in $\delta^{18}\text{O}$ across years. As can be seen in Figure 2.3, the 2002 year class had a significantly altered signature and both rivers showed similar patterns of inter-annual variation. Thus the negative coefficient for $\delta^{18}\text{O}$ from the CDA that was restricted to the Pamunkey is a result of a flipped parameter space compared to that defined by the Mattaponi CDA. Because the magnitudes of the coefficients are nearly identical and the trends in inter-annual variability are similar for those analyses that included both rivers together (Figure 2.3), these two rivers experienced the same trends in $\delta^{18}\text{O}$ variation across years.

Cross-validation classification accuracies of juveniles based on geochemical signatures in otoliths ranged from 72-100%, with an average classification accuracy of 91% (Table 2.4). Errors were almost exclusively restricted to a single fish from a given river. The exception to this pattern was among fish from the Mattaponi and Pamunkey Rivers, which showed moderate error rates between tributaries and among years. However, nearly all of these misclassifications were to the adjacent tributary and classification to the York River combined was high. There were no misclassifications between year classes of fish from the Hudson River, indicating strong inter-annual differences in geochemical signatures of juvenile otoliths between the 2000 to 2001 year classes.

Table 2.4 Cross-validation summary from the quadratic discrimination function analysis run on the complete set of juvenile signatures ($n = 420$). Groups are categorized by river of origin and collection year. Reported values are percent classification and numbers of individuals (in parentheses) assigned to each location and year indicated by columns. Accurate classifications to group of origin are shown on the diagonal; accuracies sum to 100% across a row for a given source group. Blanks indicate no classifications

River ^a Year	Exe 2001	Con 2001	Hud 2000	Hud 2001	Del 2000	Sus 2000	UpC 2000	Pot 2000	Rap 2000	Mat 2000	Mat 2001	Mat 2002	Pam 2000	Pam 2001	Pam 2002	StC 2000	Alt 2000
Exe 2001	96 ₍₂₇₎	-	-	-	-	-	4 ₍₁₎	-	-	-	-	-	-	-	-	-	-
Con 2001	-	100 ₍₂₈₎	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hud 2000	-	-	92 ₍₂₅₎	-	-	-	4 ₍₁₎	4 ₍₁₎	-	-	-	-	-	-	-	-	-
Hud 2001	-	-	-	100 ₍₂₈₎	-	-	-	-	-	-	-	-	-	-	-	-	-
Del 2000	-	-	5 ₍₁₎	-	85 ₍₁₈₎	5 ₍₁₎	-	-	5 ₍₁₎	-	-	-	-	-	-	-	-
Sus 2000	-	-	5 ₍₁₎	-	-	95 ₍₁₉₎	-	-	-	-	-	-	-	-	-	-	-
UpC 2000	-	-	-	-	-	-	100 ₍₂₉₎	-	-	-	-	-	-	-	-	-	-
Pot 2000	-	-	-	-	-	-	-	100 ₍₂₃₎	-	-	-	-	-	-	-	-	-
Rap 2000	5 ₍₁₎	-	-	-	-	-	-	-	95 ₍₂₀₎	-	-	-	-	-	-	-	-
Mat 2000	-	-	-	-	-	-	-	-	-	89 ₍₂₄₎	-	-	11 ₍₃₎	-	-	-	-
Mat 2001	-	-	-	-	-	-	-	-	-	4 ₍₁₎	78 ₍₂₂₎	-	7 ₍₂₎	11 ₍₃₎	-	-	-
Mat 2002	-	-	-	-	-	-	-	-	-	-	4 ₍₁₎	88 ₍₂₁₎	-	-	4 ₍₁₎	-	4 ₍₁₎
Pam 2000	-	-	-	-	-	-	-	-	-	17 ₍₃₎	11 ₍₂₎	-	72 ₍₁₃₎	-	-	-	-
Pam 2001	-	-	-	-	-	-	-	-	-	-	7 ₍₂₎	-	-	93 ₍₂₇₎	-	-	-
Pam 2002	-	-	-	-	-	-	-	-	-	-	-	5 ₍₁₎	-	-	84 ₍₁₆₎	-	11 ₍₂₎
StC 2000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	96 ₍₂₅₎	4 ₍₁₎
Alt 2000	4 ₍₁₎	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4 ₍₁₎	92 ₍₂₂₎

^a River codes. Exe: Exeter River, NH. Con: Connecticut River, CT. Hud: Hudson River, NY. Del: Delaware River, NJ. Sus: Susquehanna River, MD. UpC: Upper Chesapeake Bay, MD. Pot: Potomac River, MD. Rap: Rappahannock River, VA. Mat: Mattaponi River, VA. Pam: Pamunkey River, VA. StC: Santee-Cooper River, SC. Alt: Altamaha River, GA.

2.3.2 Adult American shad

Otoliths were collected from a total of 78 adults during their spawning migration into the York River. Adults ranged in age from 4 to 8 years old and were dominated by the 1996-1998 year classes (ages 4 to 6). Significant inter-annual variability meant that the combined ground-truthed juvenile signatures from 2000 to 2002 could not be used to determine natal origins of the York River adults. However, York River juveniles separated well from all other rivers based on $^{87}\text{Sr}:^{86}\text{Sr}$ and Sr:Ca values only (Figure 2.6a). Further, $^{87}\text{Sr}:^{86}\text{Sr}$ and Sr:Ca ratios did not vary significantly among years in the York River tributaries or in the Hudson River. It was therefore assumed that adults collected in the York River could be divided into fish that were homing to their natal river and those that were spawned in a different river system based on $^{87}\text{Sr}:^{86}\text{Sr}$ and Sr:Ca ratios in otolith cores. Moreover, consistent differences in $^{87}\text{Sr}:^{86}\text{Sr}$ and Sr:Ca ratios between the Mattaponi and Pamunkey Rivers meant that the natal tributary of those adults spawned in the York River could be determined. This consistency was assessed by a DFA based on Sr:Ca and $^{87}\text{Sr}:^{86}\text{Sr}$ ratios in juveniles from the Mattaponi and Pamunkey Rivers pooled across years. High classification accuracies for the Mattaponi (67%) and Pamunkey (89%) Rivers supported the assumption of consistent differences in these two ratios between the York River tributaries. Five adults (6%) were outside the range of $^{87}\text{Sr}:^{86}\text{Sr}$ and Sr:Ca ratios from juvenile otoliths collected in the York River and were therefore classified as strays that were spawned in a different river system (Figure 2.6b). Of the remaining 73 adults whose $^{87}\text{Sr}:^{86}\text{Sr}$ and Sr:Ca signatures matched those of

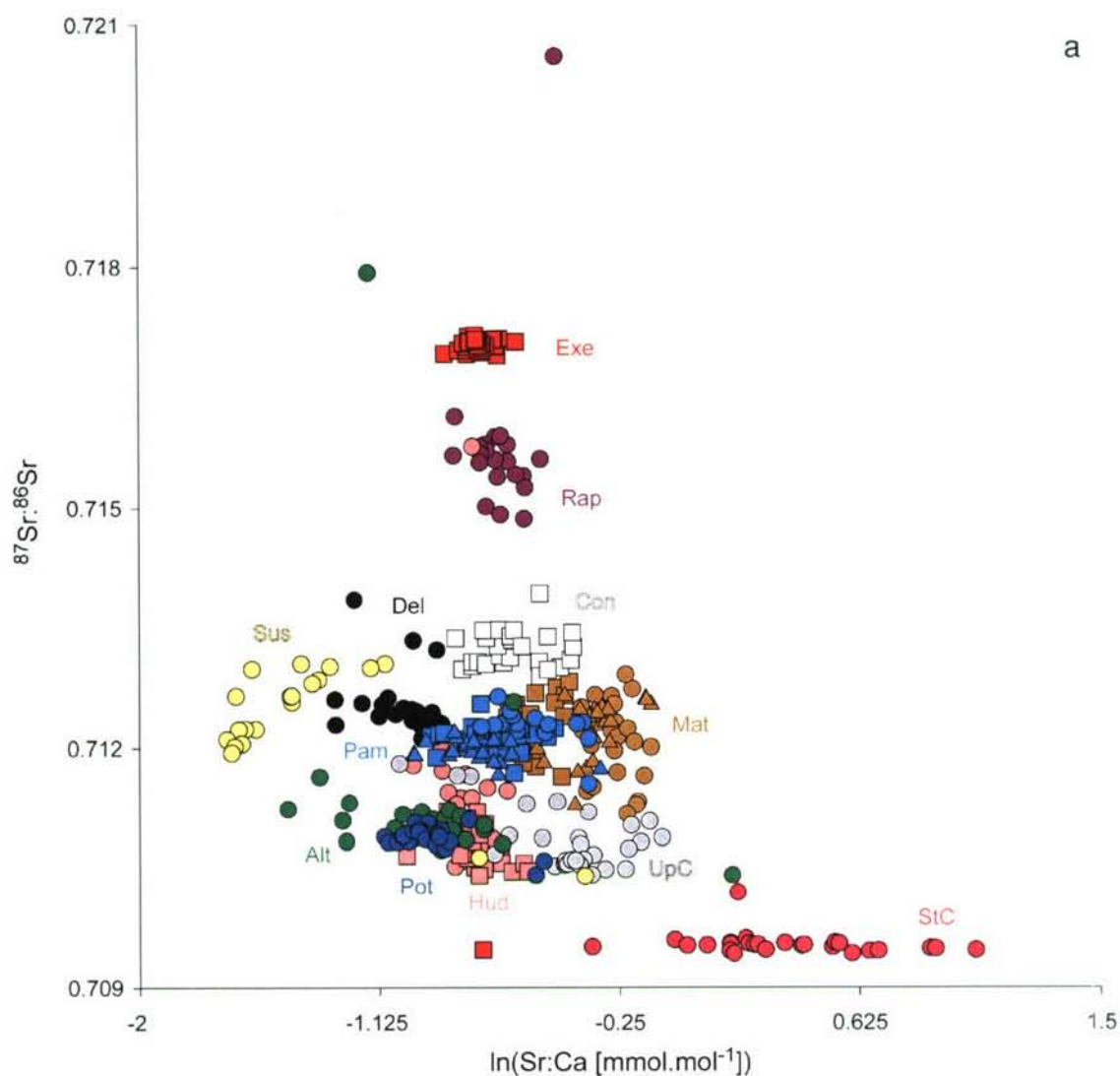


Figure 2.6a Juvenile American shad signatures for Sr:Ca and $^{87}\text{Sr}:^{86}\text{Sr}$ ratios for all fish collected in 2000 (circles), 2001 (squares) and 2002 (triangles) grouped by river of origin and year class. Sr:Ca ratios are plotted on a natural log scale for ease of visualization. River codes are described in Table 2.4.

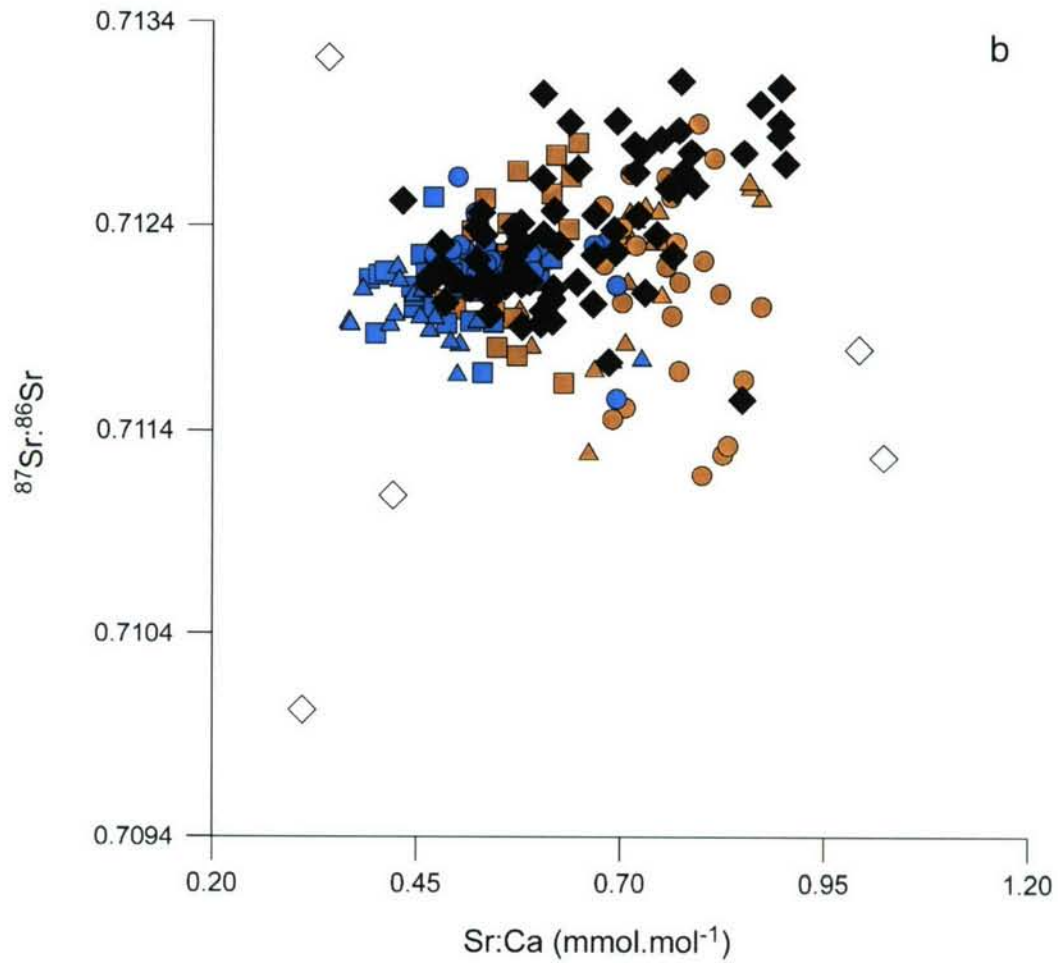


Figure 2.6b Otolith signatures from cores of York River adults of York river origin (filled diamonds) or strays from other rivers (open diamonds) plotted over juvenile signatures from the Mattaponi and Pamunkey (symbols indicate year classes as in Figure 2.5a.).

York juveniles, the MLE analysis found that an estimated $79 \pm 8 \%$ (1 SD) were spawned in the Mattaponi River and $21 \pm 8 \%$ (1 SD) were spawned in the Pamunkey River.

2.4 DISCUSSION

Geochemical signatures in juvenile American shad otoliths collected over a wide geographical range were highly distinct and specific to their river of origin. Earlier work found that elemental signatures in the otoliths of juvenile American shad were able to distinguish among the Delaware, Hudson and Connecticut Rivers (Thorrold et al. 1998b). The data demonstrate that the approach can be extended to determine natal origins of American shad spawned throughout the species range by also assaying stable C, O and Sr isotopes. Moreover, signatures are sufficiently different among rivers that the natal origins of individual fish can be identified with accuracies of 90% and higher. Natural geochemical tags in otoliths of anadromous fishes will be particularly useful for determining population affinities of individuals during ocean residency where this is currently only possible for hatchery fish that can be marked before release (e.g., Volk et al. 1999).

The source of variability in otolith geochemical signatures among rivers depends on the elemental or isotopic ratio under consideration. A strong latitudinal cline in $\delta^{18}\text{O}$ was observed, with enriched values occurring in the south and depleted values in the north. The cline mirrors latitudinal trends in $\delta^{18}\text{O}$ values of precipitation that arise due to the Rayleigh distillation process (Dansgaard 1964; Bowen 1988). Experimental work has demonstrated neither kinetic nor metabolic fractionation of $\delta^{18}\text{O}$ between otolith aragonite and ambient water, suggesting that otolith $\delta^{18}\text{O}$ values directly reflect water

$\delta^{18}\text{O}$ values, as modified by temperature (Thorrold et al. 1997; Høie et al. 2003).

Juvenile American shad therefore record $\delta^{18}\text{O}$ signatures that reflect the latitude of the watershed in which they were spawned.

Strontium isotopes in otoliths provided a powerful addition to the suite of variables used to determine natal origins of juvenile American shad. The composition of rocks within a watershed determines the $^{87}\text{Sr}:^{86}\text{Sr}$ ratio of dissolved inorganic Sr in river water. Otolith Sr reflects, in turn, the composition of the ambient water (Kennedy et al. 2000). Differences in bedrock geology among river drainages therefore generate predictable variations in otolith $^{87}\text{Sr}:^{86}\text{Sr}$ values that are likely to be stable over ecological time scales.

Multivariate geochemical signatures in otoliths of juveniles from the York and Hudson Rivers differed significantly among years. This variability was primarily driven by fluctuations in $\delta^{18}\text{O}$ values. Because otolith oxygen isotopes are deposited in equilibrium with ambient water (Kalish 1991a; Thorrold et al. 1997; Høie et al. 2003), the variability likely derived from environmental factors that altered the source or amount of water vapor that fell as rain in the watersheds. For instance, tropical storms can import isotopically heavy $\delta^{18}\text{O}$ water to higher latitudes (Cole et al. 1999). The potential for $\delta^{18}\text{O}$ to be affected by stochastic environmental effects highlights the importance of ground-truthing juvenile signatures from each cohort of interest when $\delta^{18}\text{O}$ is included in the classifying signature.

This estimate of approximately 6% of spawning adults in the York originating from other rivers supports previous estimates of mixing among populations. Several extensive tagging studies reported returns of tagged adult American shad to the river of

previous spawning (Talbot and Sykes 1958; Nichols 1960b; Melvin et al. 1986). Although these studies were unable to directly test homing to natal rivers, Melvin et al. (1986) estimated 3% of returning spawners were possible strays. Only three previous efforts involved releasing tagged juveniles in a stream to determine natal homing. Hollis (1948) reported tag recaptures within 10 miles of the release site, but the conclusions were limited by a sample size of 3 recaptured fish. Tetracycline marks in otoliths of hatchery-reared larvae allowed Olney et al. (2003) estimate that an average of 4% of marked fish caught in the James River were strays from the Pautuxent, Pamunkey, Juniata and Susquehanna Rivers. McBride et al. (2005) also used tetracycline marks to estimate a 0.001% probability of fish straying from the Susquehanna River to the Delaware River. Homing behavior has been inferred based on significant meristic, morphological and life history variation among populations (Carscadden and Leggett 1975b, 1975b; Melvin et al. 1992). Subtle but significant genetic differences in mitochondrial and microsatellite DNA sequences suggests reproductive isolation among populations and even lower straying rates than reported here (Nolan et al. 1991; Waters et al. 2000). These results add further support to the hypothesis that most American shad home to their natal river to spawn.

The dominance of adults spawned in the Mattaponi River among returning York River adults corresponds with long-term juvenile production trends in the York River. The sample was dominated by the 1996-1998 year classes (ages 4-6). Juvenile abundance indices in the river basin indicate consistently low recruitment in the Pamunkey River and relatively high recruitment in the Mattaponi River for all year classes of the returning adult spawners (Wilhite et al. 2003). Because of consistent

differences in abundances of American shad eggs and larvae in the two tributaries of the York River, it has been hypothesized that the system is dominated by contributions from the Mattaponi River population (Bilkovic et al. 2002; Wilhite et al. 2003). The results of the natal classifications based on otolith chemistry confirm that the dominance of the Mattaponi River population remains strong once fish mature. As a result, marine mortality of migrants does not appear to alter the relative abundance of these two populations and the effects of year class strength are evident during spawning events despite extensive migrations during their years at sea.

Although most mature York River adults were apparently returning to their natal river to spawn, there is evidence that this homing tendency is not preserved at the level of tributaries within a river. Subsequent to the collections of spawning adults in 2002, spawning migrants taken in the same location were used in a acoustic tagging study to determine their ultimate spawning location (Olney et al. 2006). Although there was evidence of significant handling effects, most released fish ultimately migrated to either the Mattaponi or Pamunkey Rivers to spawn. Of these migrants, 57% spawned in the Mattaponi River and 43% spawned in the Pamunkey River (Olney et al. 2006). If the natal origins of the tagged migrants in 2003 reflected the composition of the adult sample (79% Mattaponi River origin and 21% Pamunkey River origin) in 2002, the data suggest that a large proportion of tagged American shad migrating to the Pamunkey River were of Mattaponi River origin. These results indicate that although migrants home to the York River system, they do not discriminate between the two tributaries when selecting a spawning habitat. This behavior may act to subsidize the Pamunkey River population with spawners hatched in the Mattaponi River, ensuring population persistence despite

recruitment failure. Coupling data derived from otolith chemistry analyses and tagging approaches presents new opportunities to validate tributary-specific juvenile abundance indices even after extensive marine migrations. Such information is critical to effective management of these exploited and recovering populations.

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Chapter 3

CONTINENTAL-SCALE VARIATION IN OTOLITH GEOCHEMISTRY OF JUVENILE AMERICAN SHAD

Chapter submitted to *Canadian Journal of Fisheries and Aquatic Sciences* and under review as of January 29, 2007. Reprinted with permission of the publishers.

ABSTRACT

Natural geochemical markers in otoliths of diadromous fishes are powerful tools that can reveal migration patterns. This chapter investigated the ability of chemical signatures in juvenile American shad (*Alosa sapidissima*) otoliths to discriminate natal river origins on a large geographic scale. Otoliths and water samples were collected from 20 major spawning rivers from Florida to Quebec, and were analyzed for elemental (Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca) and isotope ($^{87}\text{Sr}:$ ^{86}Sr and $\delta^{18}\text{O}$) ratios. Relationships between water and otolith composition were examined for 5 rivers where both water and otoliths were sampled. Significant positive relationships were found between water and otolith Sr:Ca, Ba:Ca, $^{87}\text{Sr}:$ ^{86}Sr , and $\delta^{18}\text{O}$ ratios, but relationships were not significant for Mg:Ca and Mn:Ca ratios. The significant relationships were used to predict otolith signatures for rivers where only water was collected. Using only four chemical ratios, signatures were highly distinct among rivers, with an average classification accuracy of 93%. This is the largest assembled database of otolith signatures from habitats across a contiguous geographic range, encompassing approximately 2700 km of coastline and 19 degrees of latitude.

3.1 INTRODUCTION

The movements of diadromous fishes during oceanic residency are often poorly understood (Metcalf et al. 2002; McDowall 2003). Most anadromous species leave their natal rivers to enter marine waters relatively early in their life cycle at a size that is difficult to catch and physically mark in sufficient numbers without inducing significant handling effects. Fish may subsequently travel thousands of kilometers before returning to freshwater spawning locations (Dadswell et al. 1987; Quinn and Leggett 1987).

Groups of migrating fish collected in oceanic environments may, then, comprise individuals from numerous source populations, though the compositions of these mixed stock groups are generally unknown. Our inability to determine natal origins remains a significant problem for determining stock-specific exploitation rates in ocean-intercept fisheries, and more generally in attempts to link climate variability with changes in the distribution, growth and mortality of anadromous fish populations during the ocean phase of their life history.

While conventional tagging studies may be logistically difficult in highly dispersive marine systems, significant progress has been made using natural markers of population identities (Thorrold et al. 2002). Although rarely as unequivocal as artificial labels, natural tags avoid handling issues and, because all individuals from a population are marked, may be particularly useful when recapture rates are expected to be low. For instance, population genetic markers, including allozyme and DNA loci, have been used for mixed stock analyses of anadromous fishes collected in ocean-intercept fisheries (Brodziak et al. 1992; Brown et al. 1999). More recently, geochemical signatures in fish

ear bones (otoliths) have been successfully used to determine natal origins and are powerful tools to reveal migration patterns (Campana and Thorrold 2001). Otoliths are paired aragonitic structures in the inner ears of bony fishes and grow by the continuous accretion of metabolically inert material (Campana and Nielson 1985; Campana 1999). The composition of accreted material reflects the chemistry, temperature, and salinity of the ambient water in which the fish resided (Bath et al. 2000; Walther and Thorrold 2006). Thus, otoliths effectively record variations in environmental parameters over the lifespan of the fish. Fish that are spawned in chemically distinct habitats record these proxies of natal origin in the core region of their otoliths. Each individual from these habitats is indelibly tagged for life, making reconstructions of movement patterns possible (Thorrold et al. 2001).

American shad (*Alosa sapidissima*) are anadromous alosine clupeids native to the eastern coast of North America. Mature fish spawn in freshwater habitats from Florida to Quebec, beginning in the winter at low latitudes and progressing through the summer at high latitudes (Limburg et al. 2003). Juveniles develop in fresh water and migrate to the ocean, where they spend 3 to 7 years before returning to fresh water to spawn (Maki et al. 2001). Marine movements of American shad are known from extensive tagging efforts that described complex long-distance migrations along the North American coast (Dadswell et al. 1987). Despite the potential for significant stock mixture at sea, most American shad appear to spawn in their natal river (Waters et al. 2000; Chapter 2). There is a pressing need to understand the migratory dynamics of American shad because many populations are under moratorium and stock abundances throughout their range are at historic lows (Olney and Hoenig 2001; Limburg et al. 2003).

Otolith geochemical tags have proven useful for identifying American shad populations. Thorrold et al. (1998b) demonstrated significant variability of elemental signatures in juvenile otoliths from three major rivers on the northeast Atlantic coast. Based on a suite of elemental and stable isotope ratios, Chapter 2 reported distinct otolith signatures from 12 natal rivers. This chapter expands upon that work to include all significant spawning populations of American shad covering the entire native range of the species, approximately 2700 km of coastline and 19 degrees of latitude. Collections of otoliths and river water samples from a subset of these rivers were made to determine if ambient water concentration were correlated with otolith composition. These relationships allowed otolith chemistry to be predicted for rivers where no otoliths were collected.

3.2 MATERIALS AND METHODS

3.2.1 Field collections and otolith analyses

Juvenile American shad were sampled in 2004 from 13 rivers throughout their native range between Florida and Maine (Table 3.1). Fish were collected using push nets, seines and trawls in freshwater and upper-estuarine regions of each river prior to their downstream migration to the oceanic environment. All fish were frozen upon collection. Collections were timed to coincide with highest abundance of juveniles in each river, beginning in January at low latitudes and proceeding through October at higher latitudes. On average, 50 fish (range 18-59) from each river were included in the

Table 3.1 Juvenile American shad and water samples collected to ground-truth signatures in each spawning location; fish lengths (mean \pm standard deviation) are total lengths for the Exeter and Roanoke Rivers and fork length for all remaining rivers.

River	Juveniles (<i>n</i>)	Length (mm)	Water samples (<i>n</i>)
Miramichi	-	-	5
St. Lawrence	-	-	4
Shubenacadie	-	-	4
St. John	-	-	4
Annapolis	-	-	3
Kennebec	58	52 \pm 7	4
Exeter	41	61 \pm 8	-
Merrimack	-	-	5
Connecticut	51	58 \pm 9	5
Hudson	54	71 \pm 9	5
Delaware	57	75 \pm 9	-
Upper Chesapeake	58	72 \pm 7	-
Potomac	57	52 \pm 5	-
Rappahannock	18	60 \pm 8	-
Mattaponi	59	58 \pm 7	5
Pamunkey	-	-	5
Roanoke	57	81 \pm 10	-
Santee-Cooper	57	52 \pm 4	-
Altamaha	26	84 \pm 6	-
St. Johns	59	78 \pm 7	5

analyses. Fish were unavailable from rivers where juvenile abundance was severely reduced in 2004, e.g., the Susquehanna and Chowan Rivers.

Frozen fish were thawed, measured (fork length or total length), and dissected to remove pairs of sagittal otoliths. Otoliths were rinsed in distilled water, air-dried, and mounted on glass slides with cyanoacrylic glue. Mounted otoliths were ground to the midplane using fine-grained lapping film. Ground otoliths were examined for oxytetracycline marks indicating hatchery origin under a UV light source. Hatchery marks were detected in juveniles from the Kennebec (14%, $n = 8$), Exeter (30%, $n = 14$), and Rappahannock (39%, $n = 7$) rivers. Hatchery-marked juveniles from the Roanoke were excluded from the otoliths selected for analysis prior to processing at the laboratory. After screening for hatchery marks, one otolith from each pair was randomly chosen for laser ablation analysis. This otolith was scrubbed, sonicated in ultrapure water for 2 minutes, and triple-rinsed in a class 100 clean room.

Cleaned otoliths were analyzed for Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca, ratios on a Thermo Finnigan Element2 single collector inductively coupled plasma mass spectrometer (ICP-MS) coupled to a 213 nm laser ablation system. A 200 x 200 μm raster was ablated adjacent to the core and extending to the posterior lobe. Elemental ratios were quantified by monitoring ^{25}Mg , ^{48}Ca , ^{55}Mn , ^{86}Sr , and ^{138}Ba . Analytical methods followed those of Rosenthal et al. (1999) as modified and described in Chapter 2. A He gas stream carried ablated material to the ICP-MS where it was mixed with an Ar sample gas and a wet aerosol (2% HNO_3) supplied by a self-aspirating (20 $\text{m}\cdot\text{min}^{-1}$) PFA nebulizer in the concentric region of the quartz dual inlet spray chamber. Instrument blanks of 2% HNO_3 were run at the beginning, middle, and end of a block of

ten otoliths. A dissolved otolith certified reference material (CRM - Sturgeon et al. 2005) was used to correct for instrument mass bias (Rosenthal et al. 1999) and instrument precision was assessed using a second otolith CRM (Yoshinaga 2000). Correction procedures followed Chapter 2. External precisions (relative standard deviations) for the second CRM ($n = 134$) were 36% for Mg:Ca, 26% for Mn:Ca, 0.3% for Sr:Ca, and 1% for Ba:Ca.

After elemental ratio analysis, the same otolith was used for $^{87}\text{Sr}:^{86}\text{Sr}$ ratio analysis with a Thermo Finnigan Neptune multiple collector ICP-MS coupled to a 213 nm laser ablation system. The laser software was used to trace out a 250 x 200 μm raster centered on the nucleus, extending toward the posterior lobe of each otolith and adjacent to the raster ablated for elemental ratio measurements. Ablated material was carried by a He gas stream from the laser cell to the ICP-MS where it was mixed with an Ar sample gas and a wet aerosol in a spray chamber as described above for the elemental analyses. In addition to ^{84}Sr , ^{86}Sr , ^{87}Sr , and ^{88}Sr , counts of ^{83}Kr and ^{85}Rb were monitored to account for potential interferences on Sr isotopes (Barnett-Johnson et al. 2005; Jackson and Hart 2006). Following Chapter 2, the contribution of ^{87}Rb to ^{87}Sr intensities was removed by applying a mass bias correction to ^{85}Rb counts derived from measured $^{88}\text{Sr}:^{86}\text{Sr}$ ratios. Kr intensities were subtracted from ^{84}Sr intensities until the $^{84}\text{Sr}:^{88}\text{Sr}$ value equaled natural abundance ratios (0.006755), and that Kr value used to remove ^{86}Kr contributions to ^{86}Sr intensities. All data were normalized to a SRM987 $^{87}\text{Sr}:^{86}\text{Sr}$ value of 0.71024 based on mean $^{87}\text{Sr}:^{86}\text{Sr}$ values measured in SRM987 for a given analysis day. Mean (\pm 1 SD) values of $^{87}\text{Sr}:^{86}\text{Sr}$ values in the SRM987 ($n = 41$) and an otolith CRM ($n = 74$) run throughout the analyses were 0.71025 ± 0.00002 and 0.70916 ± 0.00002 , respectively.

True values of $^{87}\text{Sr}:^{86}\text{Sr}$ are 0.71024 for SRM987 and 0.70918 for the otolith CRM, both of which are within 1 standard deviation of mean measured ratios.

The second otolith from each juvenile was analyzed for $\delta^{18}\text{O}$ ratios using isotope ratio mass spectrometry (IR-MS). A computer-controlled micromill removed a 400 x 400 μm raster with a 75 μm drilling depth. Each milled raster was adjacent to the nucleus and extending toward the posterior lobe. The resulting powder was weighed and transferred to individual glass sample vials. Mean mass ($n = 653$) of the sampled powder was $46 \pm 15 \mu\text{g}$ (1 SD). Samples were analyzed on a Thermo Finnigan MAT252 equipped with a Kiel III carbonate device following methods outlined by Ostermann and Curry (2000). Isotopic values were reported relative to Vienna Pee Dee belemnite (VPDB) and expressed in standard δ notation. The long-term precision estimate of the mass spectrometer based on analyses of NBS19 is ± 0.07 for $\delta^{18}\text{O}$ (Ostermann and Curry 2000). All corrected chemical data for each otolith included in the analyses are provided in Appendix 3.

3.2.2 Water sample analyses

Water samples were collected from 12 rivers along the Atlantic Coast (Table 3.1). Samples were taken from between 3 and 5 locations along the mainstem of each river, ranging from just above the salt wedge to the highest region currently accessible by spawning shad, usually determined by a major dam. Collections were made between late August and early October, 2004. At each location, 50 mL of river water was collected for Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, and $^{87}\text{Sr}:^{86}\text{Sr}$ ratio analysis. Water was filtered through

0.2 μm Nalgene polytetrafluoroethylene (PTFE) filters with syringes into acid-washed low-density polyethylene (LDPE) bottles and acidified to 2% with ultrapure HNO_3 . All plastic materials used for sample collection were acid-washed prior to use. An additional 60 mL of river water for $\delta^{18}\text{O}$ ratios was filtered into glass vials and sealed with air-tight screw caps. These samples were not acidified. Samples were kept on ice in the field and refrigerated upon return to the laboratory.

Filtered and acidified samples were diluted to 10% with ultrapure water and analyzed using solution-based ICP-MS to measure ^{24}Mg , ^{43}Ca , ^{55}Mn , ^{88}Sr , and ^{138}Ba on a Thermo Finnigan Element2 single collector as described above. Liquid standards and instrument blanks of 2% HNO_3 were run every 6 samples. Instrument mass bias was corrected by using certified values of a river water standard (SLRS-4, NRC), and an internal laboratory river water standard was used to assess measurement precision. External precisions (relative standard deviations) for the laboratory standard ($n = 20$) were 0.6% for Mg:Ca, 0.5% for Mn:Ca, 0.5% for Sr:Ca, and 0.9% for Ba:Ca.

Prior to analysis of ^{87}Sr : ^{86}Sr ratios in river water, a 20 mL aliquot of each sample was evaporated to dryness, redissolved in 50% HNO_3 , and eluted through a Sr-specific cation exchange resin consisting of 18-crown-6 crown ether oligomers for Sr solvation. The sample was again evaporated to dryness and then redissolved in 1 mL of 5% HNO_3 for Sr isotope analysis using a Thermo Finnigan Neptune multiple collector ICP-MS. Data were corrected for isotopic interferences as described above for otolith analyses. The mean (± 1 SD) value of ^{87}Sr : ^{86}Sr values in SRM987 ($n = 6$) run throughout the analyses was 0.71026 ± 0.00002 , which compares favorably with the accepted value of 0.71024 for SRM987.

The $\delta^{18}\text{O}$ ratios of water samples were quantified by equilibrating 0.5 cm³ of sample with CO₂ at atmospheric pressure following methods described by Epstein and Mayeda (1953). The resulting gas was transferred from the water equilibration system to a Europa GEO 20-20 dual-inlet mass spectrometer. Method precision based on measurements of an internal standard was $\pm 0.08\text{‰}$ for $\delta^{18}\text{O}$ (Swart and Price 2002). Water data are reported relative to Vienna Standard Mean Ocean Water (VSMOW) using the standard δ notation. All corrected chemical data for each water sample included in the analyses are provided in Appendix 3.

3.2.3 Statistical analyses

The relationship between water and otolith chemistry was examined using elemental and isotopic ratios in juvenile otoliths and river water samples from the Kennebec, Connecticut, Hudson, Mattaponi, and St. Johns rivers. Linear regressions were fitted to scatterplots of mean ratios of juvenile otoliths against river water samples for all chemical signatures. To determine whether significant fractionation occurred for $\delta^{18}\text{O}$ ratios between otoliths and fresh water, the fractionation factor α was calculated, where

$$(3.1) \quad \alpha = \frac{\delta_c + 1000}{\delta_w + 1000}$$

with δ_w representing water sample $\delta^{18}\text{O}$ ratios in VSMOW averaged over all samples taken from one river, and δ_c representing otolith $\delta^{18}\text{O}$ ratios converted to VSMOW and averaged over all otoliths analyzed from one river. Conversion from VPDB to VSMOW used the following formula (Coplen et al. 1983):

$$(3.2) \quad \delta^{18}O_{VSMOW} = 1.03091 * \delta^{18}O_{VPDB} + 30.91$$

The fractionation factor α was calculated for each of the 5 rivers where both juvenile otoliths and water samples were analyzed.

Linear regression was used to predict mean otolith values based on mean water sample ratios for those rivers where no juveniles were collected. An estimate of the error about these predicted means was calculated using the equation for the standard error of the prediction error:

$$(3.3) \quad SE_{OTO} = \sqrt{s^2 + s_{\hat{y}}^2}$$

where s^2 is the estimated variance of the regression and $s_{\hat{y}}^2$ is the variance of the predicted mean otolith ratio for a given mean river ratio (Devore 2004). The standard error of the prediction error was chosen because it accounted for the greater uncertainty inherent in predicting one random variable (an otolith ratio) from another (a water ratio). The exception was $^{87}\text{Sr}:^{86}\text{Sr}$ ratios where water values were used as direct predictors of the means and standard errors of otolith $^{87}\text{Sr}:^{86}\text{Sr}$ ratios. It was assumed that any deviation from a 1:1 line between $^{87}\text{Sr}:^{86}\text{Sr}$ values in otoliths and water was due to random errors because otolith Sr compositions have been previously observed to directly reflect ambient water composition at the time of deposition (Kennedy et al. 1997; Kennedy et al. 2000). For each of the 7 rivers where no juveniles were collected, 50 fish were simulated with a normal distribution random number algorithm based on predicted otolith ratios and standard errors. A quadratic discriminant function analysis (DFA) was performed on the entire database of simulated and actual otolith signatures from 20 rivers. The DFA used a jack-knife cross-validation procedure to determine classification

accuracy and distinctness of signatures among rivers. The inclusion of hatchery-marked juveniles did not affect the results of the DFA and they were therefore pooled with non-hatchery juveniles.

3.3 RESULTS

Development of a comprehensive ground-truthed atlas of geochemical signatures in the otoliths of juvenile American shad depended on the ability to reconstruct otolith composition for those rivers where only water samples were collected. Therefore, comparisons were made between water and otolith chemistry from those rivers (Kennebec, Connecticut, Hudson, Mattaponi, and St. Johns) where both juvenile American shad otoliths and water samples were collected. Four of the 6 variables showed significant positive regression ($p < 0.01$) between water and otolith chemistry (Figure 3.1). The relation between Sr:Ca ratios (mmol.mol^{-1}) in river water ($Sr:Ca_{\text{water}}$) and otoliths ($Sr:Ca_{\text{oto}}$) was given by:

$$(3.4) \quad Sr : Ca_{\text{oto}} = 0.29 * Sr : Ca_{\text{water}} - 0.08 \quad (r^2 = 0.99).$$

The relation between Ba:Ca ($\mu\text{mol.mol}^{-1}$) in water ($Ba:Ca_{\text{water}}$) and otoliths ($Ba:Ca_{\text{oto}}$) was given by:

$$(3.5) \quad Ba : Ca_{\text{oto}} = 0.01 * Ba : Ca_{\text{water}} + 2.80 \quad (r^2 = 0.94).$$

Otolith $\delta^{18}\text{O}$ values ($\delta^{18}\text{O}_{\text{oto}}$, VPDB) accurately reflected those of the ambient water ($\delta^{18}\text{O}_{\text{water}}$, VSMOW) according to the following regression:

$$(3.6) \quad \delta^{18}O_{\text{oto}} = 1.4 * \delta^{18}O_{\text{water}} + 1.5 \quad (r^2 = 0.98).$$

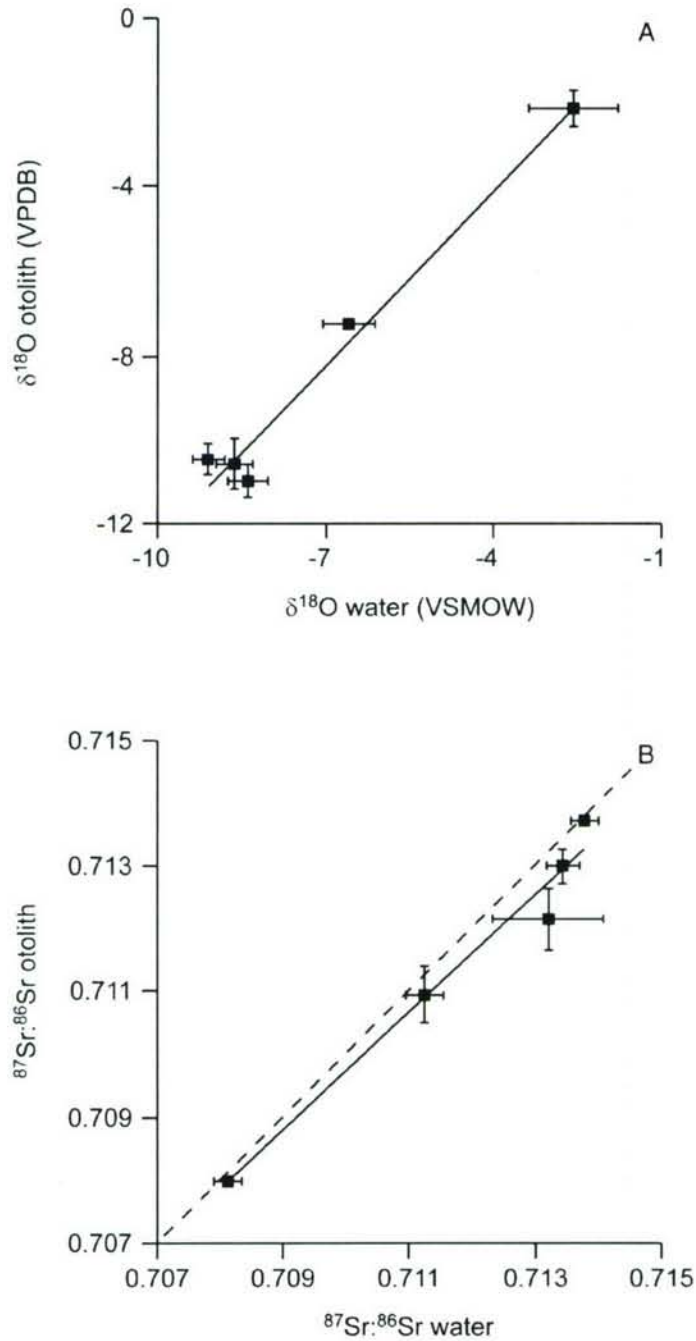


Figure 3.1 Regressions of mean \pm 1 SD otolith elemental and isotopic ratios against mean water \pm 1 SD ratios. Values are for the 5 rivers where both otoliths and water samples were collected. Regression equations are calculated using mean values. Slopes of $\delta^{18}\text{O}$, $^{87}\text{Sr}:^{86}\text{Sr}$, $\text{Sr}:\text{Ca}$ and $\text{Ba}:\text{Ca}$ ratios are statistically significant. A.) $\delta^{18}\text{O}$ ratios. B.) $^{87}\text{Sr}:^{86}\text{Sr}$ ratios. 1:1 relationship shown as dashed line.

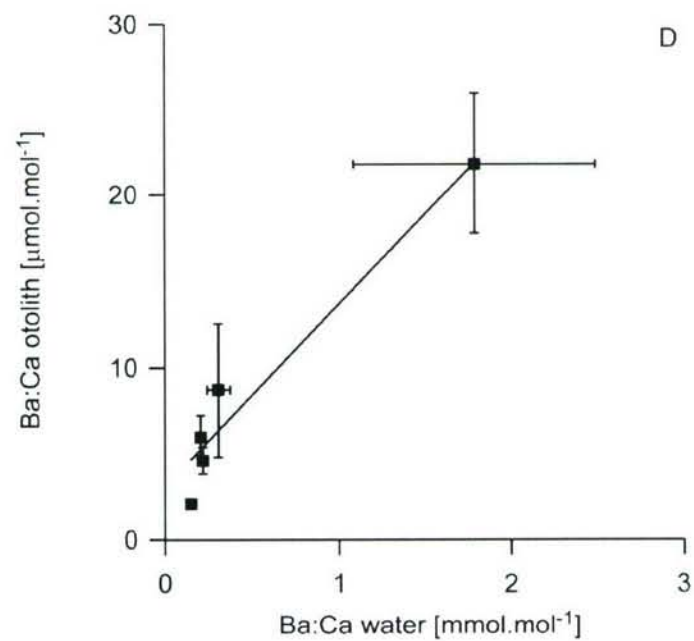
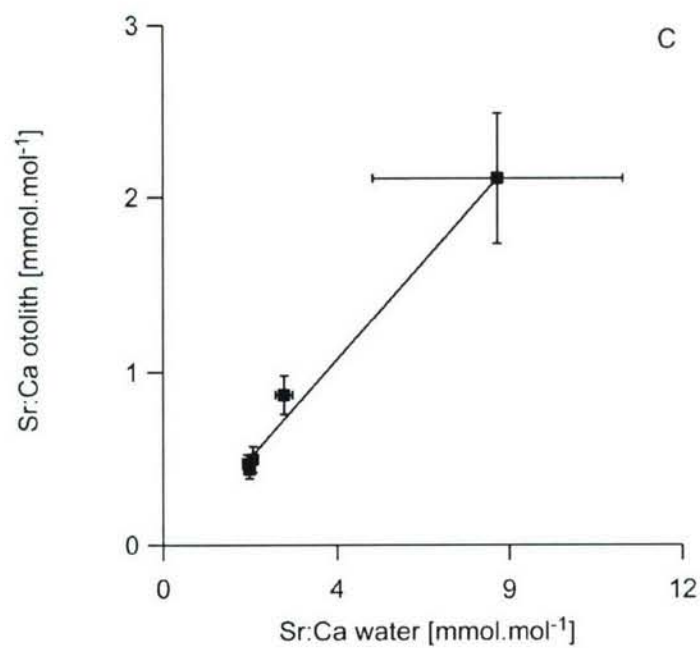


Figure 3.1 continued C.) Sr:Ca ratios. D.) Ba:Ca ratios.

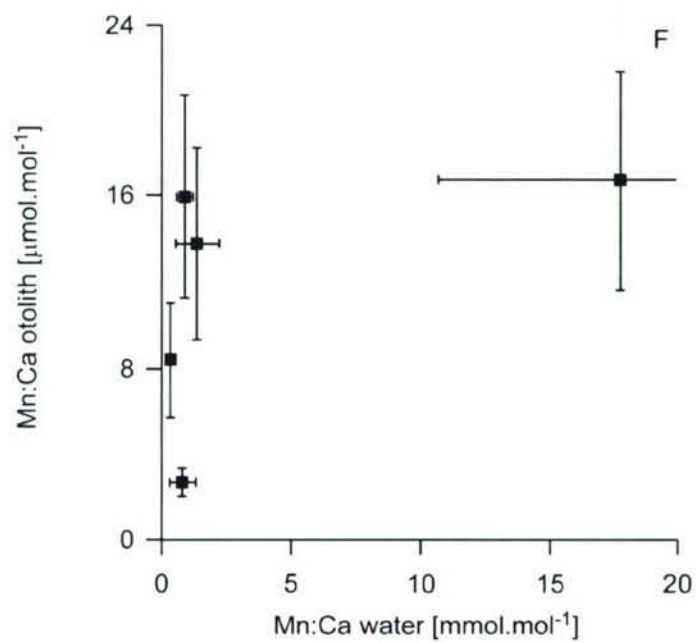
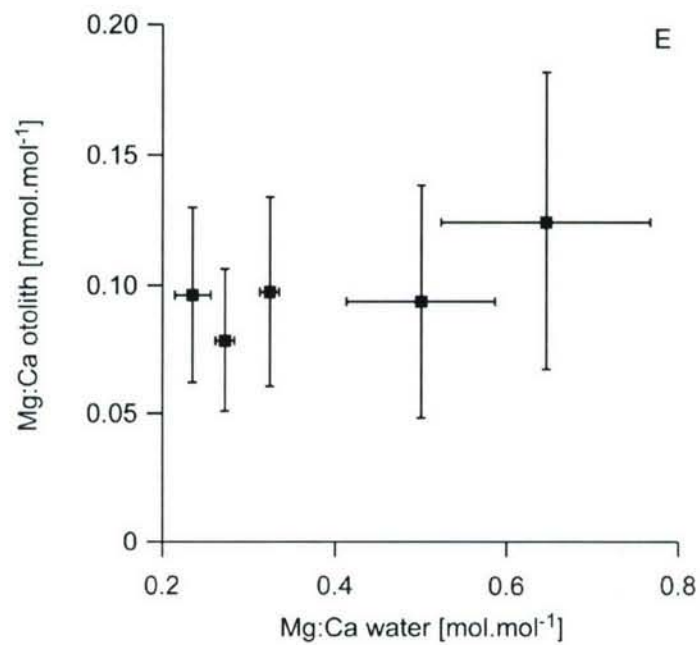


Figure 3.1 continued E.) Mg:Ca ratios. F.) Mn:Ca

Isotope fractionation factors were calculated to determine if oxygen isotopes in otoliths were deposited close to isotopic equilibrium with river waters (Figure 3.2). Water temperature was measured at the time that samples were taken for chemical analyses. Oxygen isotope fractionation factors [$10^3 \cdot \ln(\alpha)$] in the rivers ranged from 27.9 to 30.9. However, the data did not fall directly on the inorganic aragonite fractionation line. While the deviations from isotopic equilibrium were not systematic, they were clearly beyond analytical errors of the $\delta^{18}\text{O}$ measurements in otoliths and water. These deviations likely occurred because of a mismatch in measured water temperature and $\delta^{18}\text{O}_{\text{water}}$ values (sampled in late summer) and those values experienced by the fish during accretion of the material analyzed for $\delta^{18}\text{O}_{\text{oto}}$ values (accreted throughout the summer). Since α depends on water temperature and $\delta^{18}\text{O}_{\text{water}}$, mismatched values result in values that deviate from the inorganic fractionation line. The regression between water and otolith $^{87}\text{Sr}:^{86}\text{Sr}$ ratios deviated only slightly from the 1:1 line:

$$(3.7) \quad ^{87}\text{Sr}:^{86}\text{Sr}_{\text{oto}} = 0.94 \cdot ^{87}\text{Sr}:^{86}\text{Sr}_{\text{water}} + 0.04 \quad (r^2 = 0.97).$$

These data confirmed that otolith $^{87}\text{Sr}:^{86}\text{Sr}$ ratios directly reflect those of the ambient water. It was possible to predict otolith values based on water chemistry data from some, but not all, of the examined geochemical signatures. Variations in otolith Mg:Ca and Mn:Ca ($p > 0.05$) showed no detectable dependence on dissolved ratios measured in river water samples (Figure 3.1). Ratios of Mg:Ca and Mn:Ca were therefore excluded from all subsequent analyses.

Elemental and isotope ratios measured in otoliths and water samples varied significantly among rivers (Figure 3.3). There were systematic variations in $\delta^{18}\text{O}$ values along the latitudinal gradient of individual watersheds, although the pattern was less clear

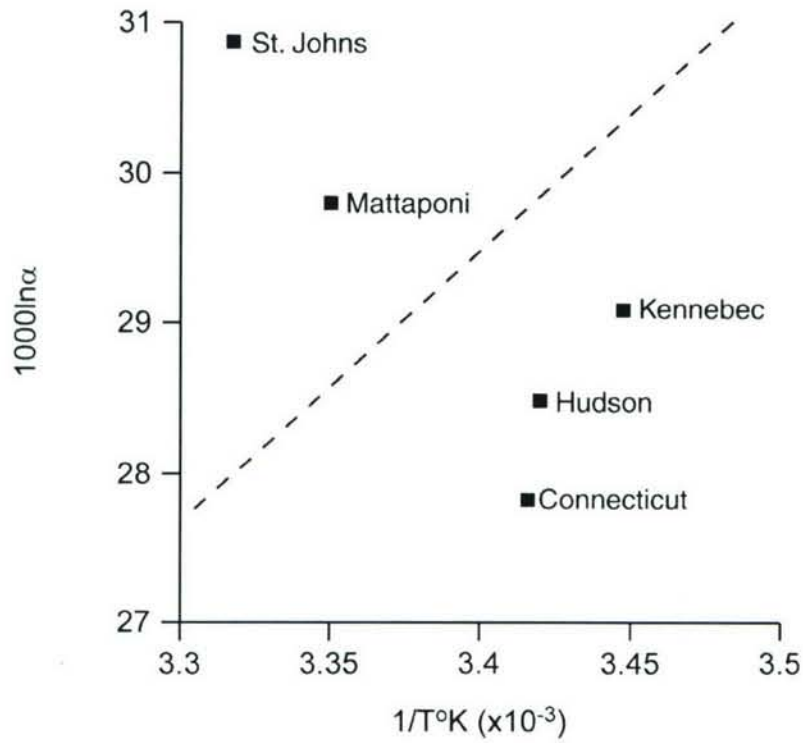


Figure 3.2 Relationship between the oxygen isotope fractionation factor $10^3 \ln(\alpha)$ and mean river water temperature measured at the time of water sample collection for the 5 rivers where both water and otoliths were collected. Each $10^3 \ln(\alpha)$ was calculated based on the mean $\delta^{18}\text{O}$ values measured in otoliths and water samples averaged within a river. The dashed line is the experimentally-determined relationship between the fractionation factor and temperature for inorganically precipitated calcite (Kim and O'Neil 1997) with a 0.6‰ enrichment adjustment for aragonite (Tarutani et al. 1969).

with the inclusion of coastal Canadian rivers. Both Sr:Ca and Ba:Ca were characterized by a lack of systematic differences among rivers and higher within-river variability than observed for $\delta^{18}\text{O}$ values. Finally, $^{87}\text{Sr}:^{86}\text{Sr}$ ratios showed lower variation among samples within an individual river than the elemental ratios, and with no obvious latitudinal gradient.

Analyses of river-specific geochemical signatures were restricted to the four elemental and isotope ratios (Sr:Ca, Ba:Ca, $\delta^{18}\text{O}$, and $^{87}\text{Sr}:^{86}\text{Sr}$) for which otolith values could be predicted from water samples in rivers where juvenile American shad were not collected. Discriminant function analysis was used to analyze measured or simulated otolith signatures in juvenile American shad collected from a total 20 rivers. Cross-validated classification accuracies ranged from 80% to 100%, with an average rate of 93% (Table 3.2). Misclassifications were generally restricted to only a few fish per river. Most misclassified juveniles were assigned to rivers in the same geographic region as their natal river. For instance, all 7 misclassified juveniles from the Shubenacadie River were assigned to the St. Lawrence River. Mean classification accuracy of simulated otoliths based on water chemistry measurements from rivers where juveniles were not collected (93%) was identical to the mean classification rate of those rivers where only juvenile otoliths were assayed.

Canonical structure coefficients indicated the relative importance of the four ratios in driving signature separation among rivers (Table 3.3). The absolute value of the coefficient indicates the relative importance of the ratio in driving combined signature separation along that variate while the sign describes the direction of the relationship. The first canonical variate was primarily driven by a strong latitudinal cline in $\delta^{18}\text{O}$

Table 3.2 Cross-validation summary from the quadratic discrimination function analysis run on signatures from actual and simulated otoliths ($n = 1003$, hatchery fish included). Reported values are percent classification and numbers of individuals (in parentheses) assigned to each location indicated by columns. Accurate classifications to group of origin are shown on the diagonal; accuracies sum to 100% across a row for a given source group. Blanks indicate no classifications.

River*	Mira	StLw	Shub	StJn	Anna	Kenne	Exet	Merri	Conn	Huds	Dela	UpCh	Poto	Rapp	Matt	Pamu	Roan	SaCo	Alta	StJs
Mira	98 ₍₄₉₎	-	-	2 ₍₁₎	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
StLw	-	94 ₍₄₇₎	6 ₍₃₎	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shub	-	14 ₍₇₎	80 ₍₄₀₎	-	-	-	-	-	-	-	-	-	6 ₍₃₎	-	-	-	-	-	-	-
StJn	2 ₍₁₎	-	-	98 ₍₄₉₎	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anna	-	-	-	-	94 ₍₄₇₎	-	-	-	-	4 ₍₂₎	-	2 ₍₁₎	-	-	-	-	-	-	-	-
Kenne	-	-	-	-	-	100 ₍₅₈₎	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Exet	-	-	-	-	-	-	100 ₍₄₁₎	-	-	-	-	-	-	-	-	-	-	-	-	-
Merri	-	-	-	-	-	-	-	100 ₍₅₀₎	-	-	-	-	-	-	-	-	-	-	-	-
Conn	-	-	-	-	-	-	-	2 ₍₁₎	90 ₍₄₆₎	-	6 ₍₃₎	-	-	-	-	2 ₍₁₎	-	-	-	-
Huds	-	2 ₍₁₎	-	-	7 ₍₄₎	-	-	-	-	83 ₍₄₅₎	-	2 ₍₁₎	6 ₍₃₎	-	-	-	-	-	-	-
Dela	-	-	-	-	-	-	-	-	-	-	93 ₍₅₃₎	7 ₍₄₎	-	-	-	-	-	-	-	-
UpCh	2 ₍₁₎	-	-	-	2 ₍₁₎	-	-	-	-	5 ₍₃₎	2 ₍₁₎	89 ₍₅₂₎	-	-	-	-	-	-	-	-
Poto	-	-	9 ₍₅₎	-	-	-	-	-	-	-	-	-	91 ₍₅₂₎	-	-	-	-	-	-	-
Rapp	-	-	-	-	-	-	-	-	-	-	-	-	-	100 ₍₁₈₎	-	-	-	-	-	-
Matt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	88 ₍₅₂₎	5 ₍₃₎	7 ₍₄₎	-	-	-
Pamu	-	-	-	-	-	-	-	-	-	-	2 ₍₁₎	2 ₍₁₎	-	-	6 ₍₃₎	86 ₍₄₃₎	4 ₍₂₎	-	-	-
Roan	-	-	2 ₍₁₎	-	-	-	-	-	-	-	-	-	-	-	2 ₍₁₎	-	96 ₍₅₅₎	-	-	-
SaCo	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2 ₍₁₎	98 ₍₅₆₎	-	-
Alta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4 ₍₁₎	8 ₍₂₎	-	88 ₍₂₃₎	-
StJs	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100 ₍₅₉₎

* River codes. Mira: Miramichi River, NB. StLw: St. Lawrence River, QB. Shub: Shubenacadie River, NS. StJn: St. John River, NB. Anna: Annapolis River, NS. Kenne: Kennebec River, ME. Exe: Exeter River, NH. Merri: Merrimack River, MA. Conn: Connecticut River, CT. Huds: Hudson River, NY. Dela: Delaware River, NJ. UpCh: Upper Chesapeake Bay, MD. Poto: Potomac River, MD. Rapp: Rappahannock River, VA. Matt: Mattaponi River, VA. Pamu: Pamunkey River, VA. Roan: Roanoke River, NC. SaCo: Santee-Cooper River, SC. Alta: Altamaha River, GA. StJs: St. Johns River, Florida

Table 3.3 Total canonical structure coefficients from the discriminant function analysis performed on actual and simulated otoliths.

Ratio	Variate 1	Variate 2
$\delta^{18}\text{O}$	0.887	0.452
Sr:Ca	0.672	0.085
$^{87}\text{Sr}:^{86}\text{Sr}$	-0.733	0.672
Ba:Ca	0.108	0.182

values, with values becoming isotopically lighter with increasing latitude (Figure 3.3A). Strontium isotopes and Sr:Ca ratios also contributed to separation among rivers on the first canonical variate. Excluding the Canadian rivers, $^{87}\text{Sr}:^{86}\text{Sr}$ ratios typically increased, while Sr:Ca ratios decreased, from low to high latitudes. Loadings on the second canonical variate were dominated by regional variation in $^{87}\text{Sr}:^{86}\text{Sr}$ ratios (Figure 3.3B), with a smaller contribution from $\delta^{18}\text{O}$ ratios. Finally, Ba:Ca ratios contributed little to signature separation along both variates.

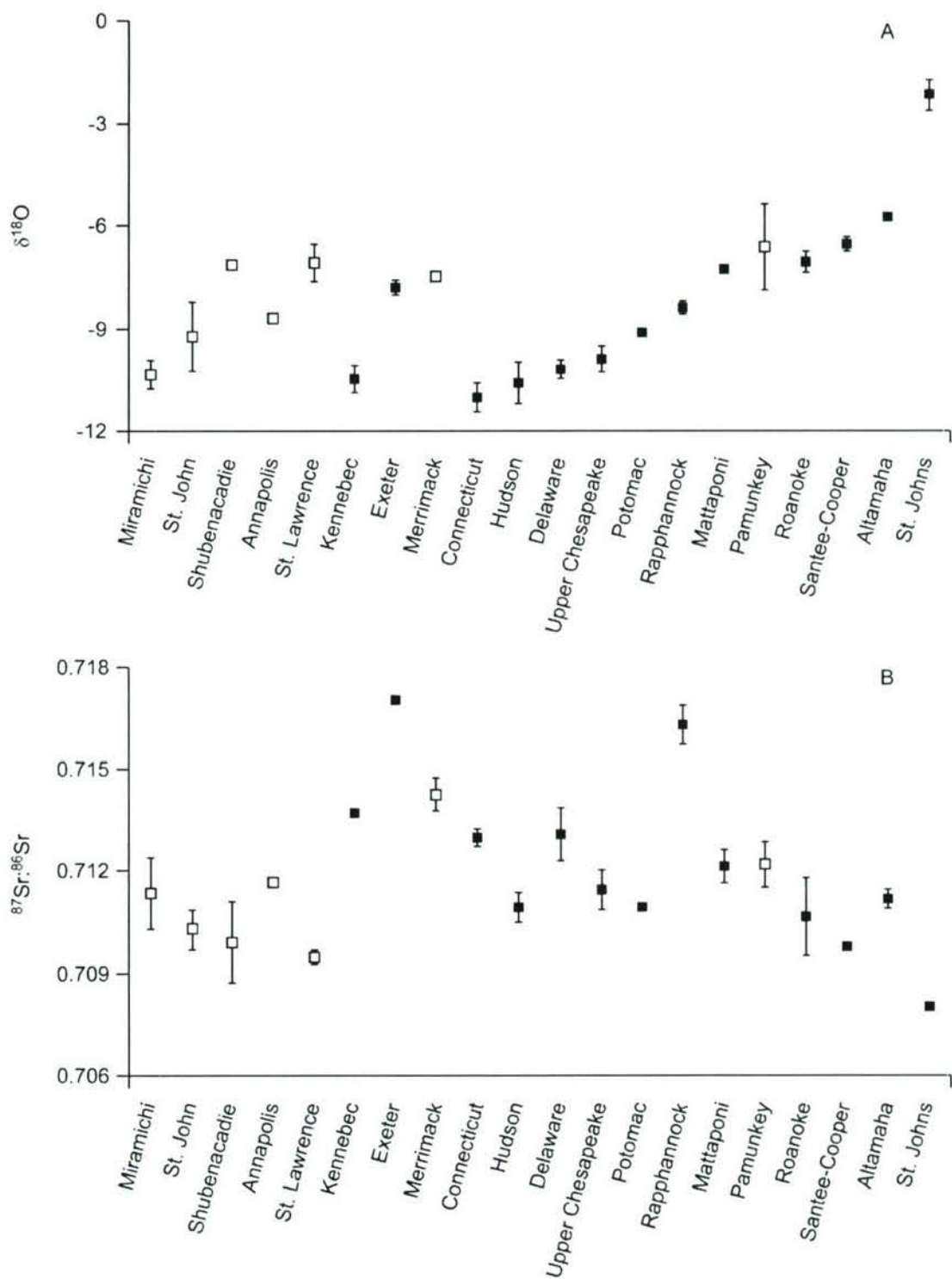


Figure 3.3 Elemental and isotopic ratios of juvenile American shad otoliths (filled symbols) and water samples (open symbols). Values are means \pm 1 SD. Water samples are displayed for rivers where no juveniles were collected. Sites are ordered from North to South. A.) $\delta^{18}\text{O}$ ratios. Units: VPDB for otoliths, VSMOW for water. B.) $^{87}\text{Sr}:^{86}\text{Sr}$ ratios.

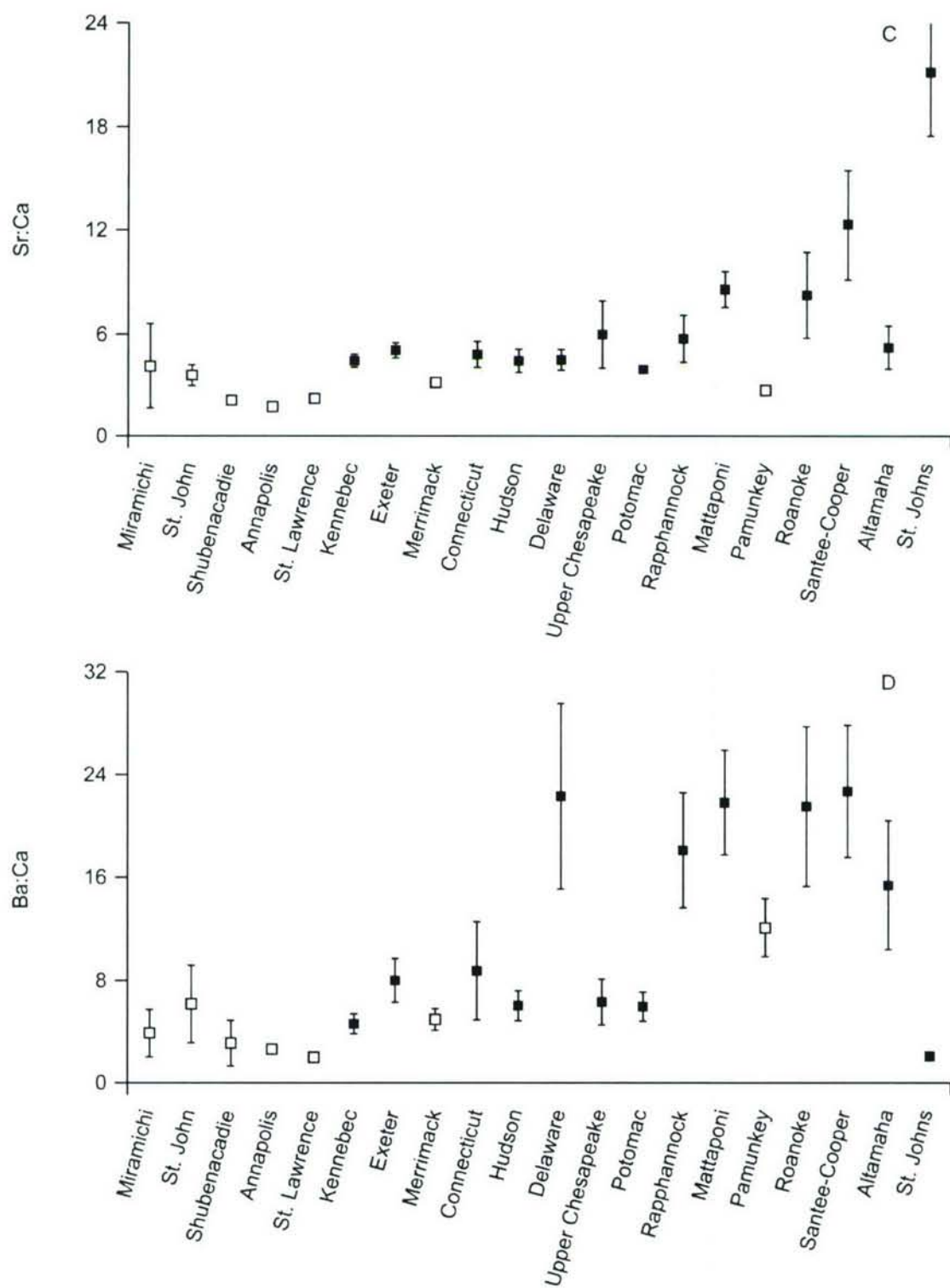


Figure 3.3 continued C.) Sr:Ca ratios. Units: mmol.mol⁻¹ for water, mmol.mol⁻¹*10 for otoliths. D.) Ba:Ca ratios. Units: mmol.mol⁻¹*10 for water, μmol.mol⁻¹ for otoliths.

3.4 DISCUSSION

Geochemical signatures in otoliths and water collected from 20 major spawning rivers along the Atlantic coast of the United States and Canada were excellent natural tags of natal origins of American shad. Cross-validation classification accuracies of individual fish based on these signatures using discriminant function analysis averaged 93% and ranged as high as 100% for 5 of the rivers. These results agree with previous, more limited work on American shad otolith signatures. Thorrold et al (1998b) were able to distinguish juvenile American shad from the Hudson, Connecticut, and Delaware rivers based on Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca in otoliths. The analyses in Chapter 2 added C, O, and Sr stable isotopes to the suite of tracers assayed by Thorrold and co-workers and were able to distinguish accurately among juvenile American shad from 12 rivers. This chapter expanded the database to include all major spawning rivers from throughout the native range of American shad, providing a comprehensive assessment of otolith signature variation for this species in 2004. Moreover, analyses were restricted to variables where otolith composition could be accurately predicted by measuring water chemistry of ambient natal environments. This, in turn, allowed geochemical signatures to be estimated in rivers where it was only possible to obtain water samples.

Water chemistry can be used to predict otolith chemistry providing there is a strong correlation between the two variables in natural environments. Experimental work has demonstrated strong effects of ambient Sr and Ba concentrations on otolith composition (Farrell and Campana 1996; Gallahar and Kingsford 1996; Bath et al. 2000; Milton and Chenery 2001; Elsdon and Gillanders 2003; Kraus and Secor 2004; Elsdon and Gillanders 2005). This chapter also found that Sr:Ca and Ba:Ca in juvenile

American shad otoliths were highly correlated with ambient levels in river waters. In contrast, neither Mg:Ca nor Mn:Ca ratios in otoliths were correlated with dissolved levels in the ambient water in this study. Limited experimental evidence has also suggested that variations in otolith Mn:Ca ratios are not related to dissolved Mn:Ca ratios (Elsdon and Gillanders 2003), while the relationship between water and otolith Mg has not been addressed experimentally. Both Mg and Mn play important roles in metabolic processes yet are toxic at high concentrations (Lehman and Joyce 1993; Rainbow 1997). Fish may regulate Mg and Mn concentrations to physiologically optimum levels, decoupling otolith composition from that of ambient waters. Regardless of the cause, the lack of a significant relationship between otolith and water composition for both Mg:Ca and Mn:Ca ratios means predictions could not be made based on water sample analyses for American shad.

Oxygen isotopes proved to be particularly useful for identifying natal origins of American shad. Otoliths from low latitude rivers recorded isotopically heavy ratios while otoliths from higher latitudes were progressively less enriched in ^{18}O . Latitudinal patterns in $\delta^{18}\text{O}$ ratios of precipitation and surface waters are driven by the Rayleigh distillation process and local mean annual temperatures (Dansgaard 1964; Bowen 1988; Kendall and Coplen 2001). The ability of $\delta^{18}\text{O}$ ratios to discriminate among rivers was less robust at high latitudes. The slope of the latitudinal cline decreased such that rivers north of the Chesapeake overlap in their $\delta^{18}\text{O}$ ratios. This pattern is not surprising because many of the northern rivers drain catchments in overlapping latitudinal regions (Benke and Cushing 2005).

The $^{18}\text{O}:^{16}\text{O}$ fractionation factor α was calculated for the 5 rivers where otoliths and water samples were collected. The logarithmic function $10^3 \cdot \ln(\alpha)$, which approximates the per mil fractionation, was 29.2 on average and comparable to values obtained by Thorrold et al (1997) in experimental settings. However, the analyses found that several of the rivers did not lie directly on the inorganic fractionation line, suggesting that either otoliths were not deposited in isotopic equilibrium or that $\delta^{18}\text{O}_{\text{water}}$ or temperature was inadequately constrained. All studies published to date, across a wide range of fish taxa, have shown neither kinetic nor metabolic fractionation of oxygen isotopes between water and otolith aragonite (Kalish 1991a, 1991b; Patterson et al. 1993; Radtke et al. 1996; Thorrold et al. 1997; Campana 1999; Høie et al. 2003). It therefore appears more likely that, by only sampling each of the rivers at a single time (albeit at a number of locations), the collected water samples did not capture seasonal variability in $\delta^{18}\text{O}_{\text{water}}$ that juvenile American shad had experienced within their natal rivers. For the Kennebec, Connecticut, and Hudson rivers, $\delta^{18}\text{O}_{\text{water}}$ values were between -8.3 and -9.1‰ as measured in late September and early October. However, Fairbanks (1982) showed that several New England rivers, including the Kennebec, Connecticut, and Hudson rivers, become progressively lighter in $\delta^{18}\text{O}$ through the summer and early fall. If $\delta^{18}\text{O}_{\text{water}}$ values near -10‰ were used, approximating values these rivers would experience in June, their $10^3 \cdot \ln(\alpha)$ values would shift upwards from around 28 and 29 to nearly 30, aligning very closely with the inorganic fractionation line. Similarly, for the southern Mattaponi and St. Johns rivers, decreasing the temperature estimate from approximately 28°C to 20 °C as experienced in early spring, the $10^3 \cdot \ln(\alpha)$ value shifts closer to the inorganic fractionation line. The present application only required that

otolith $\delta^{18}\text{O}$ values be accurately predicted by $\delta^{18}\text{O}_{\text{water}}$ throughout the sampling range. Nonetheless, the potential for seasonal variation in water chemistry and temperature should be considered when using water samples to ground-truth geochemical signatures in otoliths.

Several recent studies have suggested that Sr isotopes in otoliths may be powerful tracers of fish movements in freshwater environments (Hobbs et al. 2005; McCulloch et al. 2005). The current optimism is based on two observations. First, mass-fractionated corrected otolith $^{87}\text{Sr}:^{86}\text{Sr}$ ratios directly reflect ambient water composition (Kennedy et al. 2000). Secondly, the geological composition of bedrocks determines the $^{87}\text{Sr}:^{86}\text{Sr}$ ratio of dissolved Sr in a watershed (Palmer and Edmond 1992). River catchment geology can vary over surprisingly small spatial scales, and this in turn generates highly distinctive signatures of individual rivers or streams within a single river watershed (Kennedy et al. 2000). Developments in mass spectrometry have also helped to increase interest in otolith Sr isotope ratios. Traditional analysis by thermal ionization mass spectrometry (TIMS) requires that otolith material be dissolved, and then passed through a cation exchange resin column before making the isotope measurements on a TIMS instrument. Such a method would have been prohibitively time-consuming to analyze the 650 otoliths in this study. In contrast, laser ablation multiple collector ICP-MS provided a method for in situ Sr isotope analyses with no sample requirement beyond the preparation of an otolith sagittal section. Moreover, unlike earlier work (Milton and Chenery 2003), the precision of these measurements were similar to that obtained routinely by TIMS.

Data on habitat-specific geochemical signatures are frequently gathered over limited spatial scales (reviewed in Gillanders et al. 2001). While most efforts have examined signature variability on the order of 100-1000 km, a few investigators included samples from geographically distant locations. Collections of adult Atlantic cod (*Gadus morhua*) otoliths from the east coast of Canada and Iceland were separated by distances of approximately 4000 km (Campana et al. 1994; Campana and Gagné 1995). Proctor et al (1995) analyzed juvenile southern bluefin tuna (*Thunnus maccoyii*) otoliths from the coasts of South Africa and Australia, separated by up to 10 000 km. Significant differences in northern bluefin tuna (*Thunnus thynnus*) otolith signatures were detected between the Mediterranean Sea and the western Pacific Ocean, over 14 500 km apart (Secor and Zdanowicz 1998). These investigations assessed large-scale signature variability between widely separated and discrete habitats, analogous to the inclusion of an outgroup in genetic analyses. In contrast, variability in geochemical signatures in otoliths among habitats distributed across a large contiguous range has been rarely examined. These results confirm that the natal origins of anadromous fishes can be determined over large spatial scales and among multiple populations. Moreover, excellent discrimination was achieved with a total of only 4 variables that could be recovered from either otolith or water samples.

Attempts to identify natal origins of American shad have been made over several decades using a variety of approaches. Early efforts focused on natural variability in meristic and morphometric characters among rivers (Warfel and Olsen 1947; Fischler 1959; Nichols 1960a; Carscadden and Leggett 1975b; Melvin et al. 1992). Mitochondrial and microsatellite DNA polymorphisms showed subtle but significant variation among

fish from groups of rivers throughout the range of the species (Bentzen et al. 1989; Nolan et al. 1991; Epifanio et al. 1995; Waters et al. 2000; Nolan et al. 2003), and mtDNA polymorphisms have been used to examine the stock structure of oceanic harvests of American shad (Brown et al. 1999). However, a more definitive method for determining mixed stock composition of migrating American shad would provide information vital to the effective management of this fully exploited species. Stock abundances are at a fraction of their historic highs (Limburg et al. 2003) with many under moratorium (Olney and Hoenig 2001). While an ocean intercept fishery is currently closed, any decision to reopen will need to consider the potential mortality that may be exerted on individual stocks. The dynamic and variable marine movements of American shad could be explored effectively by using the ground-truthed database that has been generated to identify natal origins of migrants.

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Chapter 4

ORIGINS OF IMMATURE ANADROMOUS FISH IN THE MARINE ENVIRONMENT: NATURAL GEOCHEMICAL TAGS APPLIED TO MIXED-STOCK ANALYSIS

ABSTRACT

Migration patterns of immature fishes in the marine environment are generally unknown yet crucial aspects of life history strategies of highly migratory species. To determine stock composition of marine-phase anadromous American shad (*Alosa sapidissima*), this chapter employed a natural tag approach to identify natal origins using geochemical signatures in their otoliths (earbones). One-year-old fish were captured along the Maine coast and the Bay of Fundy from spring through summer, 2005. Otolith Sr:Ca, Ba:Ca, $\delta^{18}\text{O}$, and $^{87}\text{Sr}:^{86}\text{Sr}$ ratios were compared to an atlas of ground-truthed signatures from 20 natal rivers throughout their spawning range from Florida to Quebec. Samples were consistently dominated by fish from only two or three rivers, while all other stocks, including southern rivers, were present in minor proportions or completely absent. The composition of immature fish was significantly different from that previously reported for tagged adults, suggesting American shad exhibit complex and stage-specific migratory behavior after their transition to the marine environment.

4.1 INTRODUCTION

A basic problem in the study of migratory species is the identification of natal origins. This apparently straightforward question can be difficult to answer in practice, particularly for species that travel large distances and breed in numerous discrete locations. Traditional approaches to tracking migrants apply artificial tags or marks to organisms at some stage of their life cycle. The migratory route of the individual is inferred once the tag is recovered from a marked individual. Artificial tags have grown in sophistication with the advent of satellite monitoring capabilities, and tracking of organisms across large spatial scales is now possible (Block et al. 2005). Yet tags have inherent disadvantages that limit their use in at least some situations. An artificial tag can only be applied once the organism is large enough to carry it without significantly altering mortality or behavior. A large number of tags must be applied to ensure sufficient recovery for analysis, especially for abundant and wide-ranging species. Finally, artificial tags can only identify movements subsequent to their application. Because many species disperse from their natal habitats long before an artificial marker can be applied, origins can rarely be determined. Genetic analyses offer some solutions to these problems, but they are not a panacea. Using genetic data to track migratory movements requires sufficient sequence variability to distinguish populations. Samples are often pooled across large geographical regions in order to detect sequence divergence, thereby obscuring migratory variability at the population or subpopulation level (e.g., Ruzzante et al. 2000). In addition, genes that vary on evolutionary time scales are often unable to provide insight into ecological processes such as individual movement patterns. The difficulties associated with traditional tagging and genetic methods mean that for

many species movement patterns are poorly known, particularly during early life history stages.

An alternative approach employs the chemical composition of tissues as a natural tag. This method requires geographic variability in a chemical signature, such as an isotope ratio, that is subsequently incorporated into the tissue in proportion to its ambient abundance. Migratory patterns of birds and butterflies have been successfully studied using natural gradients in hydrogen, carbon, and nitrogen isotope ratios that are recorded in feather and wing keratin (Hobson et al. 1999; Chamberlain et al. 2000; Cherel et al. 2000; Rubenstein et al. 2002). The composition of fish ear bones (otoliths) has been particularly useful in tracking movement patterns because they are metabolically inert, continuously accrete material in increments that provide a chronological record of otolith deposition, and their concentrations of some elements and isotopes reflect that of ambient water (Campana 1999; Bath et al. 2000; Walther and Thorrold 2006). Thus a chemically distinct signature in a natal habitat is recorded in the core of an otolith for life, allowing natal origins and movement patterns to be reconstructed since birth (Thorrold et al. 2001).

The natural tag properties of otoliths from American shad (*Alosa sapidissima*) were used to determine natal origins of immature migrants in the marine environment. American shad are anadromous alosine clupeids, spawning in freshwater habitats from Florida to Quebec (Limburg et al. 2003). All stocks are thought to follow common migratory routes in the marine environment, with significant mixing of populations on their summer feeding grounds in the Bay of Fundy (Dadswell et al. 1987). Previous efforts to determine stock composition of coastal migrants used both traditional tagging

methods (Dadswell et al. 1987) and genetic sequence analysis (Brown et al. 1999). Both approaches analyzed stock compositions of adult American shad, and the marine movements of immature fish after their emigration from fresh water have not been previously addressed. This work drew on an atlas of geochemical signatures in otoliths of juvenile American shad and water samples collected in 20 rivers throughout their spawning range to parameterize natural tags from all major potential source populations (Chapter 3). By comparing signatures from otolith cores of one-year-old migrants with the ground-truthed juvenile database, natal origins of migrating fish were identified.

4.2 MATERIALS AND METHODS

4.2.1 Otolith collections and analyses

American shad were collected in the spring along the coast of Maine and in the summer in Minas Basin in 2005 (Figure 4.1). Fish were collected in the spring by trawl surveys along coastal regions of Maine that took place from May 3 to June 7, beginning in the south and proceeding northward. Trawls were conducted by the Maine Department of Marine Resources using a modified shrimp net with a 1-inch stretch mesh liner in the cod end, and nets were deployed according to a stratified random sampling design (Sherman et al. 2005). Fish were frozen and returned to the laboratory where they were thawed, measured for forklength (mm), and dissected to remove their sagittal otoliths. Fish between 100-180 mm forklength were retained, thereby restricting the analyses to fish from the 2004 year class (Leim 1924; Dadswell et al. 1984). An average of 5 fish per trawl date from the appropriate size range was collected (Table 4.1), except for one

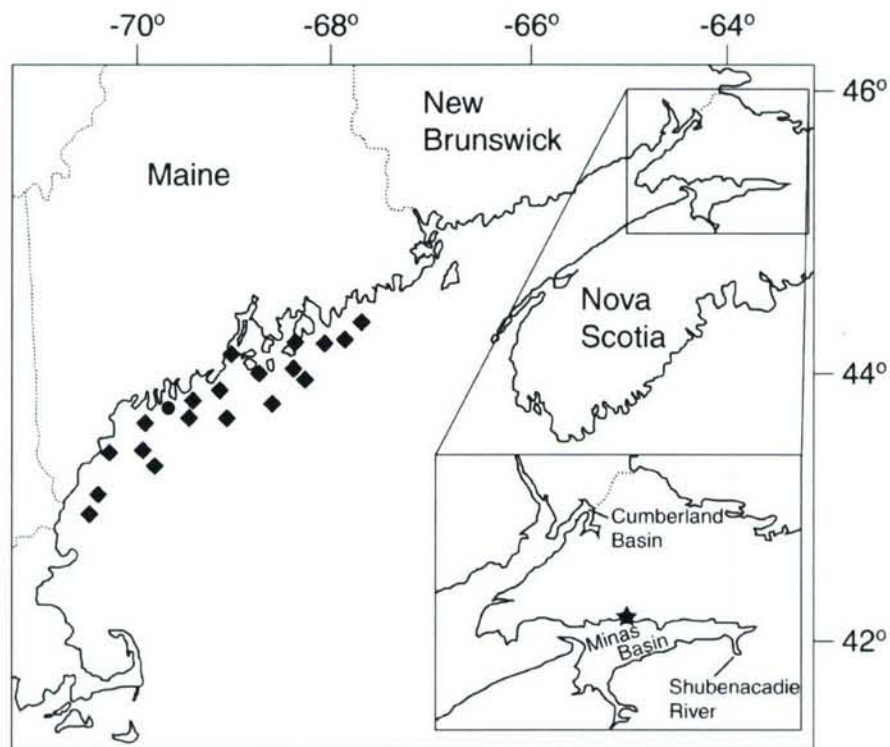


Figure 4.1 Collection locations of American shad sampled in 2004. Trawl surveys (diamonds) collected fish along the coast of Maine in the spring. The largest trawl collection (circle) of 77 fish occurred on 12 May. Summer collections were made at a weir (star) in Minas Basin.

Table 4.1 Collection data for fish sampled daily in spring trawl surveys along the coast of Maine in 2005 with along with mean (± 1 SD) forklengths, average daily trawl latitude and longitude, and average maximum trawl depths; the large trawl (LT) of 12 May is indicated.

Trawl date	<i>n</i>	Forklength (mm)	Latitude (N)	Longitude (W)	Depth (m)
3 May	8	144 \pm 16	42.99	-70.48	92
4 May	11	141 \pm 16	43.12	-70.39	79
5 May	10	137 \pm 12	43.34	-69.81	68
6 May	1	125	43.43	-70.28	62
10 May	2	136 \pm 15	43.45	-69.94	139
11 May	6	136 \pm 12	43.64	-69.92	66
12 May (LT)	77	136 \pm 16	43.77	-69.68	57
13 May	17	132 \pm 15	43.81	-69.43	78
14 May	11	152 \pm 15	43.69	-69.47	113
16 May	6	137 \pm 21	43.88	-69.16	57
17 May	4	133 \pm 6	44.14	-69.05	38
18 May	12	141 \pm 13	43.68	-69.09	113
19 May	4	148 \pm 6	43.78	-68.63	123
20 May	17	126 \pm 8	44.00	-68.76	72
30 May	1	135	44.03	-68.41	85
31 May	3	143 \pm 2	43.95	-68.30	104
1 June	5	124 \pm 11	44.22	-68.39	40
3 June	5	138 \pm 25	44.21	-68.09	59
6 June	6	133 \pm 11	44.24	-67.88	69
7 June	14	136 \pm 12	44.37	-67.72	63

large collection of 77 fish on 12 May (Large Trawl collection, hereafter referred to as LT). Subsequent to the spring trawl surveys, American shad were collected at a commercial herring weir on the northern shore of Minas Basin near Five Islands, Nova Scotia. Fish with forklengths between 100-180 mm were collected from the weir at each low tide of the semidiurnal cycle. Fish were measured and sagittal otoliths dissected on site. An average of 10 fish per day was captured in the weir (Table 4.2), with the largest collection of 66 fish occurring on 22 July (Large Weir collection, hereafter referred to as LW). Fish were collected through early August, after which they were no longer present in the weir.

Dissected otoliths were mounted and ground to the midplane with fine-grained lapping film. Ground otoliths were examined for oxytetracycline marks under a UV light source to detect hatchery marks. No marks were detected in any of the analyzed fish. After screening for hatchery marks, one otolith from each pair was randomly chosen for laser ablation analysis and cleaned in a class 100 clean room.

The cleaned otolith was analyzed on a Thermo Finnigan Neptune multiple collector inductively coupled plasma mass spectrometer (ICP-MS) coupled to a 213nm laser ablation system. Material was ablated from a 250 x 250 μ m raster centered on the nucleus of each otolith. Methods follow Chapter 2 with the following modifications. During each ablation pass, the instrument cycled between monitoring three sets of monitored isotopes: 1.) ^{83}Kr , ^{84}Sr , ^{85}Rb , ^{86}Sr , ^{87}Sr , and ^{88}Sr were monitored simultaneously for 3 seconds, 2.) ^{48}Ca was monitored for 1 second, and 3.) ^{138}Ba was monitored for 1 second. By cycling through the sets of monitored isotopes, the method quantified ratios of Sr:Ca, Ba:Ca, and ^{87}Sr : ^{86}Sr with a single ablated raster on the core of

Table 4.2 Collection data for fish sampled daily in the summer of 2005 at the Minas Basin weir with mean (± 1 SD) forklengths; the large weir collection (LW) on 22 July is indicated.

Collection date	<i>n</i>	Forklength (mm)
27 June	12	158 \pm 15
28 June	3	143 \pm 7
29 June	15	153 \pm 14
30 June	2	151 \pm 6
1 July	5	153 \pm 13
2 July	1	145
3 July	2	139 \pm 13
4 July	5	138 \pm 2
6 July	4	143 \pm 4
7 July	1	140
11 July	8	147 \pm 12
12 July	12	145 \pm 14
13 July	5	151 \pm 10
14 July	11	148 \pm 15
15 July	11	149 \pm 14
16 July	16	150 \pm 11
18 July	14	146 \pm 13
20 July	10	147 \pm 13
21 July	7	148 \pm 18
22 July (LW)	66	153 \pm 14
24 July	12	142 \pm 9
25 July	10	152 \pm 16
26 July	4	155 \pm 16
2 August	2	155 \pm 7

each otolith (e.g., McCulloch et al. 2005). Instrument blanks of 2% HNO₃ were run at the beginning and end of a block of ten otoliths and used to correct for background intensities of the monitored elements.

A dissolved otolith certified reference material (CRM - Sturgeon et al. 2005) was used to correct for instrument mass bias (Rosenthal et al. 1999) and instrument precision was assessed using a second otolith CRM (Yoshinaga 2000) as described in Chapter 2. External precisions (relative standard deviations) for the second CRM ($n = 96$) were 1.8% for Sr:Ca and 1.5% for Ba:Ca. Corrected values (mean \pm 1 SD) of Sr:Ca and Ba:Ca in the second CRM were 2.94 ± 0.05 mmol.mol⁻¹ and 2.23 ± 0.03 μ mol.mol⁻¹, respectively. These values were each within one standard deviation of average values obtained for Sr:Ca (2.95 mmol.mol⁻¹) and Ba:Ca (2.24 μ mol.mol⁻¹) using the same CRM during single collector ICP-MS analyses of juvenile otoliths and water samples to create the atlas.

Corrections for interferences of ⁸⁷Rb and ⁸⁶Kr on ⁸⁷Sr and ⁸⁶Sr, respectively, were made as outlined in Jackson & Hart (2006) and Chapters 2 and 3. All data were normalized to a NIST CRM SRM987 ⁸⁷Sr:⁸⁶Sr value of 0.71024 based on mean ⁸⁷Sr:⁸⁶Sr values measured in SRM987 for a given analysis day. The mean (\pm 1 SD) value of ⁸⁷Sr:⁸⁶Sr values in the SRM987 ($n = 40$) run throughout the analyses was 0.71026 ± 0.00003 , which is within 1 standard deviation of the true value of SRM987 (0.71024). Periodic measurements of an aragonitic marine sclerosponge ($n = 9$) yielded a mean (\pm 1 SD) value of 0.70916 ± 0.00001 , which is close to the global marine ⁸⁷Sr:⁸⁶Sr value (0.70918).

The second otolith from each fish was analyzed for $\delta^{18}\text{O}$ ratios using isotope ratio mass spectrometry (IR-MS). A computer-controlled micromill removed a 400 x 400 μm

raster centered on the nucleus with a 75 μ m drilling depth. Mean mass ($n = 458$) of the sampled powder was $61 \pm 10 \mu\text{g}$ (1 SD). Samples were analyzed on a Thermo Finnigan MAT252 equipped with a Kiel III carbonate device following methods outlined by Ostermann and Curry (2000). Isotopic values were reported relative to Vienna Pee Dee belemnite (VPDB) and expressed in standard δ notation. The long-term precision estimate of the mass spectrometer based on analyses of NBS19 is ± 0.07 for $\delta^{18}\text{O}$ (Ostermann and Curry 2000). All corrected chemical data for each otolith included in the analyses are provided in Appendix 4.

4.2.2 Statistical analyses

The geochemical signatures of one-year-old migrants were compared with signatures from known-origin juveniles and water samples collected the previous year from 20 rivers along the Atlantic coast of the United States and Canada. Water samples were used to predict otolith samples where no juveniles were collected (see Chapter 3). River-specific signatures based on Sr:Ca, Ba:Ca, $\delta^{18}\text{O}$, and $^{87}\text{Sr}:^{86}\text{Sr}$ ratios in actual and predicted juvenile otoliths were highly distinct, with an average cross-validated classification accuracy of 93%. The two most important natal signature discriminators were $\delta^{18}\text{O}$ and $^{87}\text{Sr}:^{86}\text{Sr}$ ratios (Figure 4.2). The 20 stocks in the juvenile database represent the majority of extant spawning biomass, including all stocks that are most likely to be present in the mixed sample.

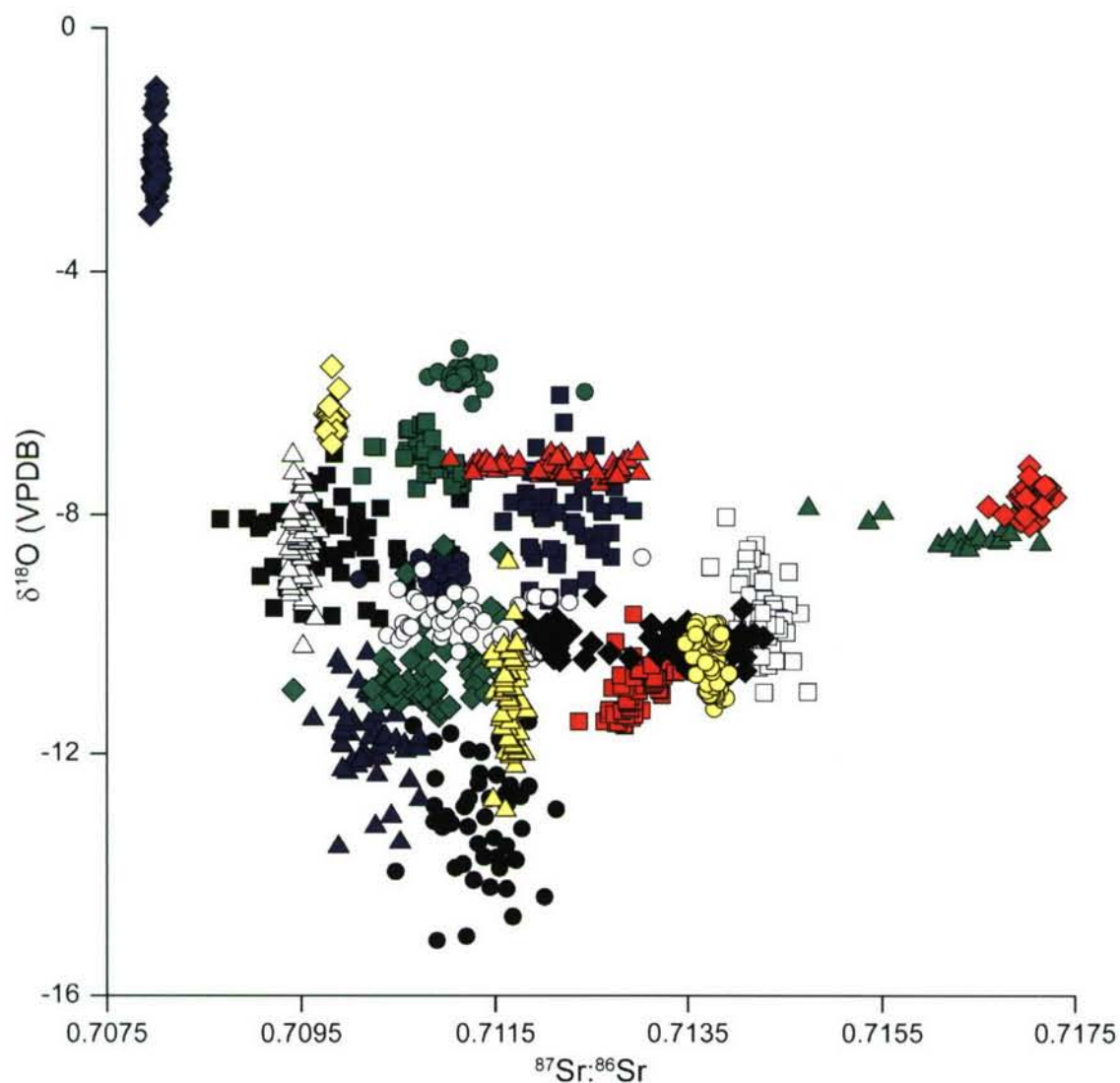


Figure 4.2 River-specific signatures of $^{87}\text{Sr}:^{86}\text{Sr}$ and $\delta^{18}\text{O}$ ratios based on juvenile otoliths and water samples collected in 2004. Each symbol represents the signature from one actual or simulated fish. Rivers represented are: Miramichi ●, St. Lawrence △, Shubenacadie ■, St. John ▲, Annapolis △, Kennebec ○, Exeter ◆, Merrimack □, Connecticut ■, Hudson ◆, Delaware ◆, Upper Chesapeake ○, Potomac ●, Rappahannock ▲, Mattaponi ▲, Pamunkey ■, Roanoke ■, Santee-Cooper ◆, Altamaha ●, and St. Johns ◆. Data from Chapter 3.

Signatures from one-year-old migrants captured in the Maine trawl surveys and Minas Basin (Figure 4.3) were plotted with those from known-origin juveniles. The majority of core signatures were within the ranges of values defined by the ground-truthed juvenile signatures. A few signatures fell outside these ranges and likely originated from sources not included in the juvenile database. These fish were excluded from estimates of migrant stock compositions to reduce potential biases in the estimation procedure.

Stock compositions were assessed using the maximum-likelihood estimation program Integrated Stock Mixture Analysis (ISMA) (Campana et al. 1999). The algorithm estimates proportions of unknown samples that derive from populations parameterized by the reference atlas of geochemical signatures but does not identify origins of individual fish. The method is similar to maximum-likelihood analyses of genetic or other biological markers (Millar 1987; Utter and Ryman 1993; Ruzzante et al. 2000; Méthot et al. 2005). Prior to analysis, each elemental and isotope ratio was examined for deviations from normality. Because normal probability plots of all four ratios showed only moderate departure from normality and transformations did not significantly alter the distributions of residuals, untransformed data were used in all analyses. Stock compositions were analyzed separately for the migrants collected from the Maine trawl surveys ($n = 209$) and the weir collections in Minas Basin ($n = 232$). Changes in stock compositions over the course of the Maine trawls were assessed by partitioning the collections into three groups: 1) Fish collected in the early spring from 3 May to 16 May ($n = 68$), excluding LT fish; 2) Fish collected in the late spring from 17 May to 7 June ($n = 68$); and 3) LT fish only (12 May, $n = 73$). Collections from the Minas Basin weir were similarly partitioned: 1) Fish collected in the early summer from

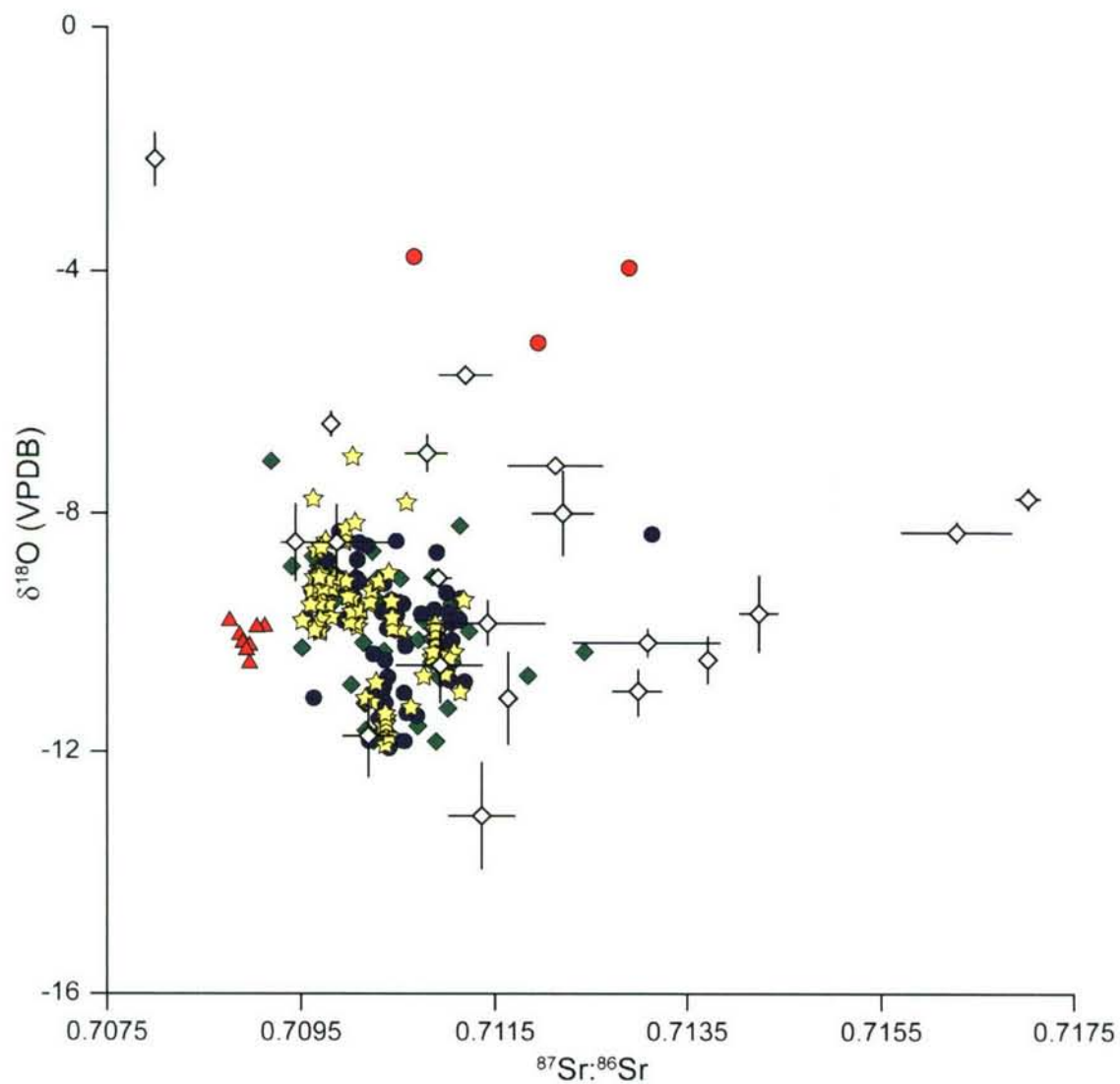


Figure 4.3 A.) Natal signatures of fish collected in spring Maine trawl surveys (filled symbols) over mean (± 1 SD) values of each source river (\diamond). Group 1 (\bullet) and Group 2 (\blacktriangle) fish were excluded from composition analysis. Collections occurred in early spring (\bullet), late spring (\blacklozenge), and on 12 May (\star).

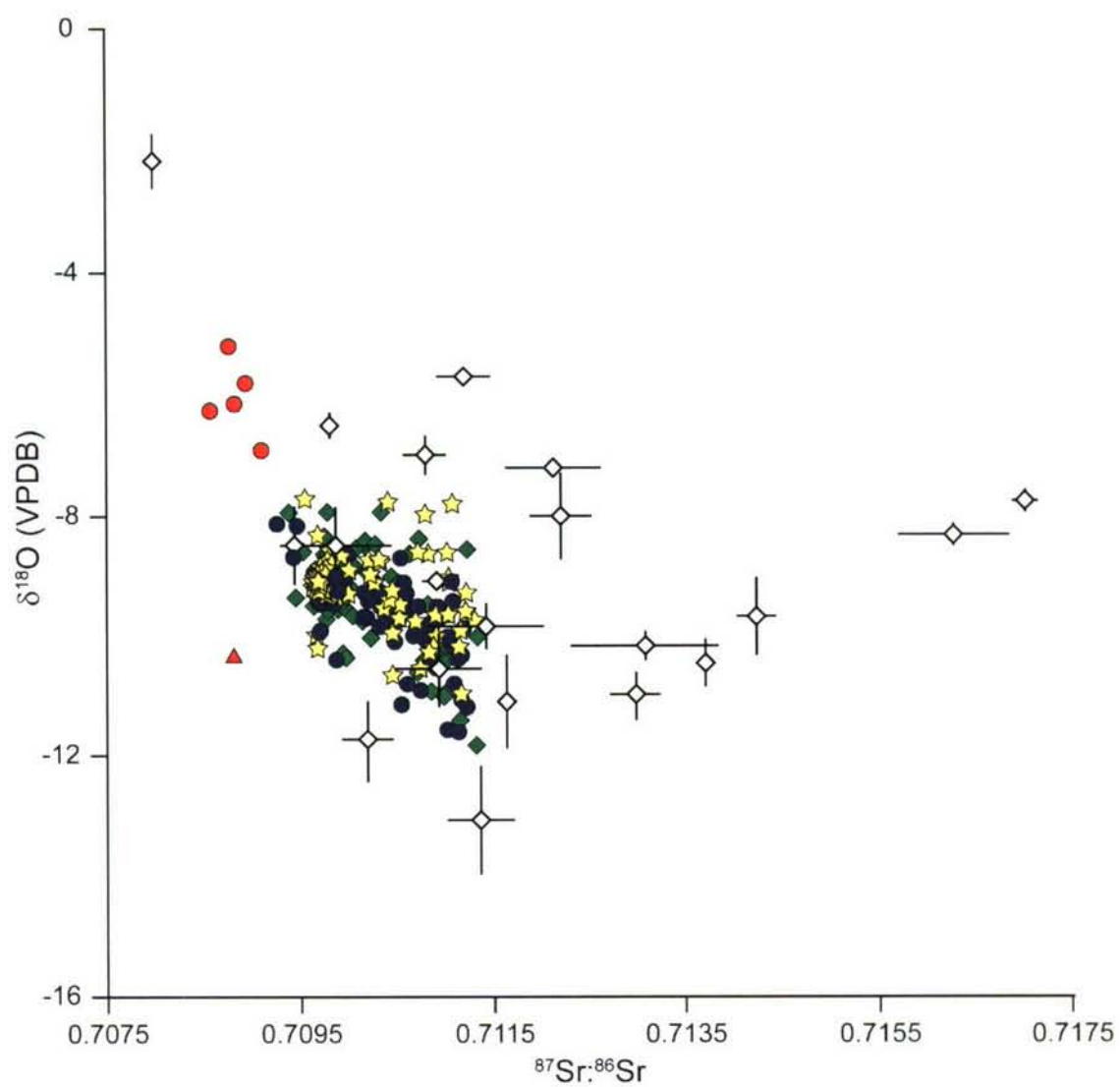


Figure 4.3 B.) Natal signatures of fish from summer Minas Basin weir collections. Fish in Group 3 (●) and an additional solitary fish (▲) were excluded from composition analysis. Collections occurred in early summer (●), late summer (◆), and on 22 July (★)

27 June to 14 July ($n = 86$); 2) Fish collected in the late summer from 15 July to 2 August ($n = 81$), excluding LW fish; and 3) LW fish only (22 July, $n = 65$). Trends in size with capture date were examined for Maine and Minas Basin migrants (Figure 4.4).

RESULTS

The majority of fish collected in the Maine trawls and the Minas Basin weir came from only two or three rivers. Stock compositions of the combined Maine trawls were dominated by fish from the Shubenacadie and Hudson rivers (Table 4.3). Small proportions (<5%) of the pooled sample originated from the St. Lawrence, St. John, Merrimack, and Connecticut rivers. Fish were only present in minor proportions from the upper Chesapeake, the Potomac and Santee-Cooper rivers, which were the only southern rivers represented. All other stocks were absent in the trawl surveys. Stock compositions of the combined Minas Basin weir collections were similarly dominated by fish from the Shubenacadie and Hudson rivers (Table 4.3). However, fish from the Potomac River were the third most abundant stock in the mixture, representing 17% of all Minas Basin weir fish. There was less diversity in the Minas Basin samples compared to the Maine trawl surveys, with only small numbers of fish from the St. Lawrence and St. John rivers and all other stocks absent from the mixture.

The spring and summer collections were each partitioned into early, late, and large collections to determine temporal variability in composition. Variability within a season was generally low. Larger differences in composition were observed between spring and summer collections. For instance, Hudson River fish made up over half of the

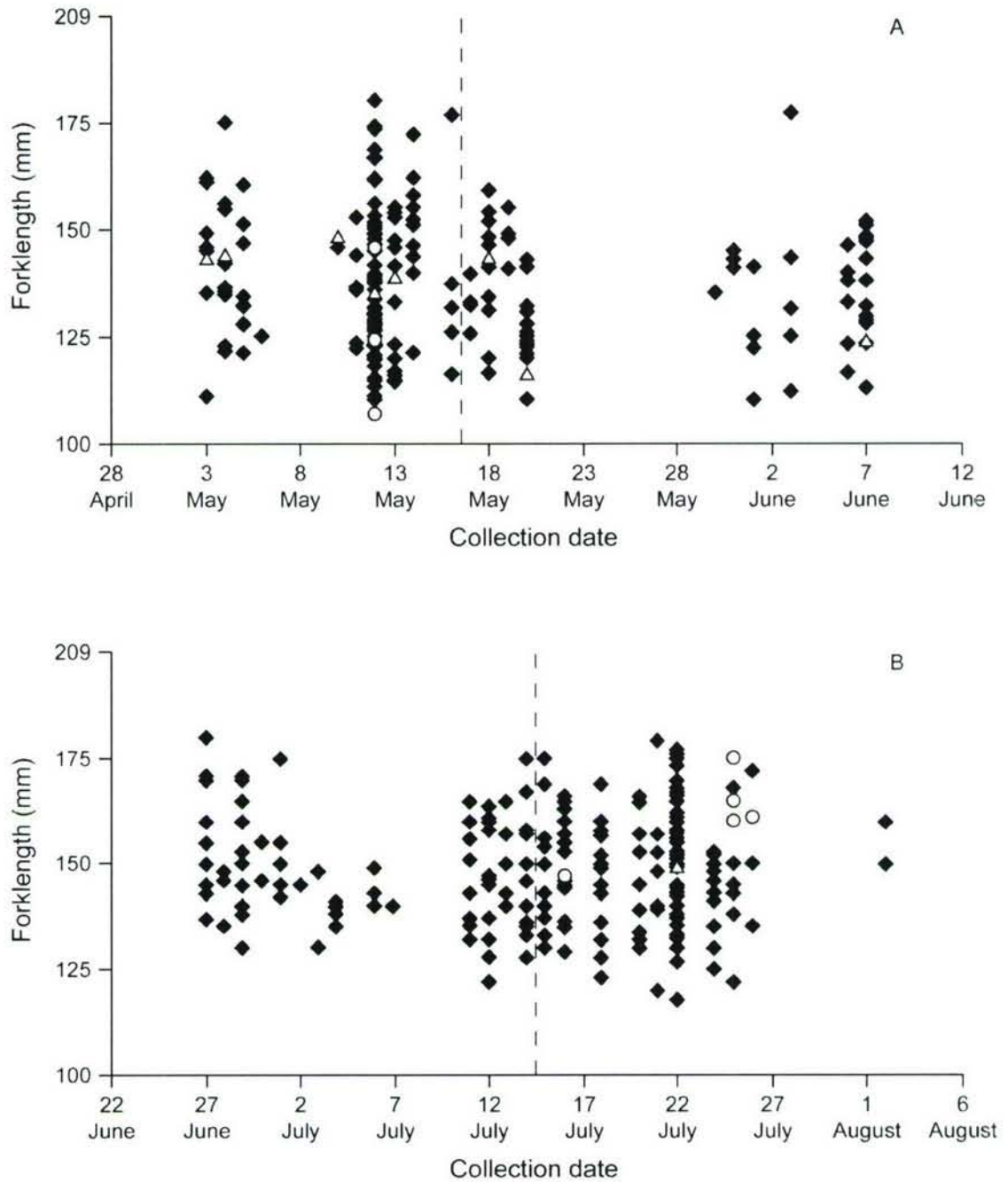


Figure 4.4 Forklength versus capture date for all fish from A.) Spring Maine trawl surveys and B.) Summer Minas Basin weir collections. Symbols for fish with signatures that did not correspond to source rivers are described in Figure 4.2. Dashed lines indicate the division of trawls into early and late collections each season.

Table 4.3 Percent composition from each source river in combined and partitioned mixtures of immature migrants from spring Maine trawl surveys and summer Minas Basin weir collections; large spring trawl (LT) and summer weir (LW) collections were each analyzed in isolation.

River	Spring				Summer			
	All	Early*	Late [†]	LT	All	Early [‡]	Late [§]	LW
Miramichi	-	-	-	-	-	-	-	-
St. Lawrence	0.3	0.2	0.3	0.2	0.3	0.5	0.2	0.3
Shubenacadie	41.3	29.0	38.6	56.7	56.4	42.7	69.2	58.1
St. John	5.4	3.4	7.5	5.6	0.3	-	1.1	-
Annapolis	-	-	-	-	-	-	-	-
Kennebec	-	-	-	-	-	-	-	-
Exeter	-	-	-	-	-	-	-	-
Merrimack	0.5	1.5	-	-	-	-	-	-
Connecticut	0.1	-	0.9	-	-	-	-	-
Hudson	44.2	55.9	41.0	35.4	25.4	32.6	24.0	15.6
Delaware	-	-	-	-	-	-	-	-
Upper Chesapeake	1.7	0.5	2.9	-	-	-	-	-
Potomac	5.6	9.6	7.3	0.7	17.7	24.2	5.5	26.1
Rappahannock	-	-	-	-	-	-	-	-
Mattaponi	-	-	-	-	-	-	-	-
Pamunkey	-	-	-	-	-	-	-	-
Roanoke	-	-	-	-	-	-	-	-
Santee-Cooper	1.0	-	1.5	1.4	-	-	-	-
Altamaha	-	-	-	-	-	-	-	-
St. Johns	-	-	-	-	-	-	-	-
<i>n</i>	209	68	68	73	232	86	81	65

*3 May to 16 May (excluding LT). [†]17 May to 7 June. [‡]27 June to 14 July. [§]15 July to 2 August (excluding LW)

collections in early spring and became progressively less abundant through the spring and summer. Correspondingly, Shubenacadie River fish became more abundant with time, contributing nearly 70% of fish to late summer collections. The Potomac River also contributed more fish in the summer than in the spring. Stock diversity also changed with time, with spring collections more diverse than summer collections. The southernmost stock present in the mixtures, the Santee-Cooper, was only detected in late spring.

In addition to examining temporal variability, the two large daily collections of Maine trawl (LT) and Minas Basin weir (LW) fish were analyzed in isolation. These analyses detected altered stock compositions in these large groups of fish compared to the smaller collections from surrounding dates. The spring LT collection had a higher proportion of Shubenacadie River fish than other early spring trawls and contained the only Santee-Cooper River fish detected in early spring collections. The summer LW collection contained a much higher proportion of Potomac River fish than all other late summer collections. The size of the collection therefore appeared to have some effect on stock composition, although the relative dominance of the detected stocks was not significantly altered.

A small number of fish from Maine trawls and the Minas Basin weir had signatures that did not match those in the juvenile atlas. Maine trawls collected 8 fish with low $^{87}\text{Sr}:^{86}\text{Sr}$ ratios and $\delta^{18}\text{O}$ values close to -10‰ (Group 1, Figure 4.3A) and 3 fish with moderate $^{87}\text{Sr}:^{86}\text{Sr}$ ratios and $\delta^{18}\text{O}$ values between -4‰ and -5‰ (Group 2, Figure 4.3A). The fish in Group 1 were smaller, with forklengths between 110-150 mm and all but one were collected before 21 May 2006 (Figure 4.4A). The fish in Group 2 were less

than 150 mm forklength and were all collected in the largest trawl on 12 May 2006 (Figure 4.4A). Minas Basin migrants included 5 fish with low $^{87}\text{Sr}:^{86}\text{Sr}$ ratios and $\delta^{18}\text{O}$ values between -5‰ and -7‰ (Group 3, Figure 4.3B), all of which were between 149 and 180 mm forklength and arrived after 16 July 2006 (Figure 4.4B). A solitary fish with low $^{87}\text{Sr}:^{86}\text{Sr}$ and a depleted $\delta^{18}\text{O}$ ratio was also excluded (Figure 4.3B). Although a few of these excluded fish had $\delta^{18}\text{O}$ values indicating southern origin, including them would not have significantly altered the composition estimates since they were present in such low numbers. There was no trend in size with collection date for fish from either Maine trawl surveys or the Minas Basin weir (Figure 4.4).

4.4 DISCUSSION

A natural tag approach was used to classify immature migrating fish in the marine environment according to their natal origins. Mixed-stock compositions of migrants collected over 4 months during their movements at sea were dominated by relatively few stocks. The Hudson and Shubenacadie rivers contributed the majority of fish to the sampled mixtures, and this trend persisted over time and between locations. The Potomac River also contributed significantly to the mixtures, with the most substantial proportion of Potomac River fish present in early summer Minas Basin collections. There was a notable lack of population diversity, with at least half of the potential contributing stocks not represented. Moreover, what little diversity was present in the spring collections declined over time, with only 5 out of a potential of 20 stocks represented in late summer collections.

Several large northern populations were noticeably absent from the samples. Aside from the Shubenacadie River, fish from all other extant Canadian stocks were either present in small numbers or were not detected. In addition, most rivers in the northeast United States contributed little to the mixtures. For instance, Connecticut River fish were present in only a few early spring collections in small numbers. The lack of fish from these northern rivers was surprising given their large historical population sizes. One possible explanation for their absences could be that northern rivers have experienced recent declines in year-class strength. The Connecticut River spawning population was estimated at approximately 350,000 fish in 2004, the lowest estimate in 9 years. In addition, juvenile abundance indices indicated relatively small year classes from 1995 to 2004 (T. Savoy, personal communication). The absence of Connecticut River fish may therefore be due to these depressed stock abundances. However, the Hudson River population has experienced comparable drops in abundance indices, and 2004 was not a strong year class (NYSDEC 2005). Yet, despite reduced juvenile abundances, Hudson River fish made up a substantial proportion of the mixed assemblages. Year-class strength therefore does not appear to account entirely for different contributions of northern stocks to the samples.

Southern and mid-latitude stocks were also generally absent from the mixtures, and many Chesapeake Bay stocks were not present in any collections. The Potomac River was the only significant contributor from the Chesapeake Bay region, accounting for up to a quarter of collected fish. While Potomac River stock abundance has been low for several decades, stocking efforts have enhanced the population and juvenile abundance in 2004 was the largest recorded to date by monitoring surveys (Sadzinski and

Jarzynski 2006). The strong 2004 year class may account for the significant numbers of Potomac River fish in the samples. Stocks from south of Chesapeake Bay were also poorly represented in the mixtures, and no fish from Florida were detected. Although their origins could not be positively identified, some of the fish that were excluded from the mixture composition analysis could have originated from southern rivers. Because of a strong latitudinal gradient in $\delta^{18}\text{O}$ ratios recorded in American shad otoliths, as reported in Chapters 2 and 3, the origins of the excluded fish with isotopically heavy $\delta^{18}\text{O}$ ratios were likely between Virginia and Georgia. However, even if included in the mixture composition analyses, they would represent approximately 2% of all collected fish, and the proportion of southern fish present overall would remain small.

The stock compositions of immature migrants in Minas Basin differ substantially from those reported for older age classes. Dadswell et al. (1987) compiled data from several decades of tagging studies on American shad throughout the western Atlantic. Summer feeding aggregations in the upper Bay of Fundy contained individuals from stocks throughout their entire range, including Florida. Fish from rivers north of Cape Cod were proportionally more abundant in the early summer, while southern stocks dominated in July and August. Of adult migrants tagged in Cumberland Basin, approximately 34% were recaptured in rivers south of Cape Hatteras (Region 1), 44% were recaptured between Cape Hatteras and Cape Cod (Region 2), 15% were recaptured in Bay of Fundy rivers (Region 3), and 7% were recaptured in the Gulf of St. Lawrence (Region 4). Dividing the composition estimates in this chapter into similar regions, on average 47% of one-year-old migrants were from Region 2 and 52% were from Region 3, while less than 1% were from Regions 1 and 4, each. Moreover, Regions 2 and 3 were

each overwhelmingly represented by a single river, indicative of the much lower stock diversity in these mixtures in this chapter compared to the adult data reported by Dadswell et al. (1987).

Previous tagging studies focused on migratory movements of American shad between 3 and 6 years old. Younger year classes are typically not tagged because of their higher natural mortality rates and the difficulties inherent in affixing tags to smaller fish without impairing behavior or survival. These difficulties require larger numbers of young fish to be tagged to ensure sufficient returns for mark-recapture analysis. Artificial tags are able to track movements only subsequent to tag application, with natal origins inferred indirectly. Although artificial tags work well for analysis of adult movement patterns, the spatial dynamics of the youngest year classes have been more successfully investigated with approaches such as otolith chemistry. The combination of traditional and geochemical techniques reveals the complexity of anadromous fish migrations in marine environments. Fish may follow substantially different migratory pathways from one life history stage to the next. A paradigm of American shad migration patterns is that individuals from all stocks throughout their range move through the upper Bay of Fundy, capitalizing on high local summer productivity and food availability (Dadswell et al. 1987). While this pattern was observed for mature fish, the results in this chapter show a different compositional pattern for immature migrants. Processes such as environmental variability, year class strength, and stock-specific behavior may collectively influence mixed-stock composition for these early life history stages. Inter-annual variability in composition was not addressed by this study, but temporal and ontogenetic changes in migratory behavior at sea are likely. The absence of southern stocks in the mixtures

suggests that American shad undertake long-distance migrations only after they reach some minimum size and such movements are energetically favorable (Weihs 1984; Roff 1991).

There are significant management implications of American shad movements and mixing in the marine environment. An assessment of mixed-stock compositions of coastal harvests off Maryland and Virginia based on mitochondrial DNA variation found significant variation in the contributions of individual stocks to the mixtures (Brown et al. 1999). This variation was both geographical and temporal suggesting dynamic and unpredictable changes in the presence of specific stocks in harvests, with many stocks represented by numbers disproportionate to their population sizes. Such dynamism limits the ability to manage coastal mixed-stock fisheries without allowing unsustainable mortality of the most vulnerable stocks. This work shows that the migratory habits of immature American shad are similarly complex, and managers of American shad should consider the potential impact of mixed-stock fisheries on early year classes. Increased mortality of immature migrants in mixed-stock fisheries has the potential to significantly alter year-class strength, restructure demographics, and further limit recovery of depleted populations. Combining results from studies employing traditional tags, morphometrics, DNA analysis, and now natural chemical tags in otoliths reveals the complex nature and stage-specific patterns of American shad migrations in the marine environment.

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Chapter 5

CONCLUSION

5.1 SYNTHESIS

This thesis capitalized on the natural tag properties of otoliths to determine natal origins of American shad during their marine and spawning migrations, and it represents the most comprehensive examination of natural tag variability for a species with a large geographical range. The findings presented here are important both for the use of otoliths as tags for anadromous fishes and for our understanding of the migratory behavior a species of considerable cultural and economic importance throughout its range.

The first step in any analysis of otolith chemistry is to quantify variation in signatures from specific locations. In Chapter 2, previous analyses of American shad otolith chemistry (Thorrold et al. 1998b) were expanded to include 13 rivers from Georgia to New Hampshire, with multi-year collections from the Hudson, Mattaponi, and Pamunkey rivers. A large suite of geochemical signatures was analyzed, and ratios of Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, ^{87}Sr : ^{86}Sr proved to be highly distinct river-specific signatures when combined. The average cross-validated classification accuracy based on these 7 ratios was 91%, with a latitudinal gradient in $\delta^{18}\text{O}$ and variability in ^{87}Sr : ^{86}Sr due to watershed geology contributing most to signature separation. These samples also afforded an opportunity to determine inter-annual variability in signatures for some rivers, a concern that is often not addressed in the otolith literature. Signatures from the Hudson, Mattaponi, and Pamunkey rivers differed significantly among years, with $\delta^{18}\text{O}$ principally responsible for the variation. This analysis shows that $\delta^{18}\text{O}$ is of great utility in distinguishing geographically separate spawning locations yet undergoes

unpredictable temporal fluctuations. Caution is therefore necessary when employing an isotope ratio such as $\delta^{18}\text{O}$ that is subject to this kind of temporal variability. When possible, signatures should be assessed for each year-class of interest, and classifications of unknown origin fish should be made using ground-truthed signatures from the same cohort.

In order to assess signature variability from all major stocks of American shad in their native range, Chapter 3 expanded on Chapter 2 and included 20 rivers from Florida to Quebec in the ground-truthed database. This task was accomplished by collecting juvenile otoliths and water samples from the rivers of interest. For the 5 rivers where both otoliths and water samples were collected, regressions between their respective chemical compositions showed which elemental and isotope ratios varied predictably in otoliths according to water chemistry. Although Sr:Ca, Ba:Ca, $\delta^{18}\text{O}$, and $^{87}\text{Sr}:^{86}\text{Sr}$ showed significant positive relationships between water and otoliths, regressions of Mg:Ca and Mn:Ca were not significant. Thus for rivers where only water samples were collected, otolith chemistry was predicted for Sr:Ca, Ba:Ca, $\delta^{18}\text{O}$, and $^{87}\text{Sr}:^{86}\text{Sr}$ ratios. Despite a reduction to only four ratios, cross-validated classification accuracies from the signatures still averaged 93%. As in Chapter 2, the most important ratios in driving signature separation among rivers were $\delta^{18}\text{O}$ and $^{87}\text{Sr}:^{86}\text{Sr}$.

Once the ground-truthed databases were assembled, they were used to classify natal origins of fish at different life history stages. The degree to which mature American shad return to their natal river to spawn was quantified using Sr:Ca and $^{87}\text{Sr}:^{86}\text{Sr}$ ratios, both of which are temporally stable, to determine origins of adults. Adults returning to the York River system were captured before they moved into the Mattaponi or Pamunkey

Rivers, the two tributaries that join to form the York River. Based on the chemical signatures in the cores of their otoliths, only 6% of spawners originated from rivers other than the Mattaponi or Pamunkey. This low rate of straying among rivers is consistent with previous estimates based on meristics, morphometrics, tagging and genetics. Of those fish that came from the York River system, nearly 80% originated from the Mattaponi River and the remaining 20% from the Pamunkey River. These relative proportions were not surprising because the Pamunkey River has experienced persistent recruitment failure in recent years. In addition, subsequent acoustic tagging data on returning spawners in the York River suggest that half of the spawners move into the Mattaponi and Pamunkey rivers, each. The acoustic tagging and otolith chemistry analyses therefore suggest that a significant portion of fish spawning in the Pamunkey River are of Mattaponi River origin, thereby subsidizing the population in the Pamunkey and contributing to its persistence. Natal homing therefore appears to be accurate at the river scale but not at the tributary scale, with important implications for metapopulation dynamics.

In Chapter 4 the natal origins of immature one-year-old American shad migrating through their summer feeding grounds were estimated using the suite of Sr:Ca, Ba:Ca, $\delta^{18}\text{O}$, and $^{87}\text{Sr}:^{86}\text{Sr}$ ratios from the large database of 20 rivers. For these mixed-stock analyses, one-year-old fish from the 2004 year class were selected by size because otolith and scale-based aging methods have not been validated for the earliest life history stages of American shad (McBride et al. 2005). The probability of young-of-the-year fish spawned in 2005 attaining the lengths of fish included in the analyzed mixtures is essentially zero. Growth rates change according to the phenomenon of countergradient

variation (Conover and Present 1990), with low growth rates in the south and higher growth rates in the north (Limburg et al. 2003). Because spawning begins in December for southern stocks and occurs progressively later with latitude, varying growth rates allow fish spawned at different times to attain similar sizes at the end of the first growing season. Fish from the St. Johns River in Florida grow at 0.3 mm/day, and thus individuals spawned in December will be approximately 55 mm long in early June and 75 mm in early August. Fish from the Shubenacadie River in Nova Scotia grow at 0.8 mm/day but are spawned in May at the earliest. Shubenacadie River young of the year spawned in May would therefore reach approximately 35 mm in June and 75 mm in August. Assuming constant growth rates, none of these juveniles would reach the 100-180 mm lengths of the fish analyzed in the mixtures. The highest observed growth rate for juvenile American shad is 1.4 mm/day in the St. Lawrence River (Limburg et al. 2003). Fish spawned in May could reach 120 mm by August, and thus there is a remote possibility the small fish collected in late summer could contain young of the year from the St. Lawrence. However, the only chemical signature to exhibit significant inter-annual variability was $\delta^{18}\text{O}$, which fluctuated up to 2‰ for some rivers. Because northern rivers overlap significantly in their $\delta^{18}\text{O}$ ratios, this signature is of lesser importance in discriminating these stocks. Thus the potential inclusion of a few St. Lawrence River fish in late summer collections with different $\delta^{18}\text{O}$ ratios is not likely to be a significant source of bias.

The mixed-stock analyses determined natal origins of immature American shad collected during their residency along the coast of Maine and the Bay of Fundy. While previous artificial tagging data suggested that fish from stocks throughout their range

migrate to these northern locations in summer feeding aggregations, Chapter 4 reported that the composition of immature migrants showed a surprising lack of diversity in stock composition and little contribution from southern stocks. Mixed samples were overwhelmingly dominated by fish from the Shubenacadie and the Hudson rivers, with increasing proportions of Potomac River fish present later in the summer. Temporal and geographic variations in compositions were minimal, with an overall decrease in stock diversity in the summer Minas Basin collections. While these data could not be used to directly assess the processes driving the differences in stock compositions of these immature migrants compared to previous tagged adults, possible explanations include fluctuations in stock abundances, environmental variability, and ontogenetic shifts in migratory behavior. These forces likely act in concert to influence the distributions of individual stocks in the marine environment.

5.2 A THEORETICAL CONTEXT:

BEHAVIORAL DECISIONS IN THE MARINE ENVIRONMENT

The data presented in Chapter 4 show that the stock composition of immature American shad in the Bay of Fundy was significantly different than that reported for older fish. While the data can address neither the reason for this difference nor the potential for inter-annual variability in immature stock composition, a plausible explanation is that immature fish pursue different migratory strategies than their older counterparts. Ontogenetic shifts in behavior may be at least partly responsible for the lack of southern stocks in the mixtures. One hypothesis, then, is that American shad exhibit ontogenetic shifts in migratory behavior, with long-distance migrations occurring

later in their marine residency phase. The migration strategy of American shad would therefore be divided into three stages: 1.) A freshwater resident phase, with variable timing of entry into seawater; 2.) An immature marine phase during which growth occurs and movements are restricted; and 3.) a mature marine phase consisting of long-distance seasonal movements. The functional cause for the shift from Phase 2 to Phase 3 would be the attainment of some minimum size or energetic stores before long-distance migrations become favorable.

Is there theoretical support for this hypothesis? The interaction of energetic status, size, and migratory behavior has been explored extensively for the freshwater stages of anadromous fishes. Intra-specific variation in movement patterns of marine animals is known (Quinn and Brodeur 1991), but logistical difficulties in assessing life history parameters in the marine environment have limited the availability to gather detailed data at sea. However, theoretical models of migratory behavior developed for freshwater stages and terrestrial species can be reasonably applied to the marine phase of anadromous fish movements.

A key concept in life history theory is the ontogenetic niche shift (Werner and Gilliam 1984). This concept derives from the observation that as the body size of an organism increases, patterns in resource use, predation risk and the influence of abiotic forces change accordingly. The niche that an individual inhabits thus shifts over time, either continuously or discretely depending on the development patterns of the organism. Werner and Gilliam (1984) argue that these shifts occur such that organisms maximize their growth rates or energy accumulation and minimize their mortality risk at each size. This translates to the simple rule that to maximize fitness, organisms should minimize the

ratio μ/g at each size, where μ is the mortality risk and g is the growth rate. This rule can be placed in the context of switching between habitats, since habitats 1 and 2 have their own μ/g ratios and residency in one habitat would be more favorable than the other at a given size. This decision to switch habitats is not fixed and operates dynamically with the organism responding to alterations in μ/g , which in turn could be influenced by environmental stochasticity, density-dependent interactions, and other predictable or unpredictable processes. In the context of American shad migrations, the decision to undertake a long-distance migration could depend on a μ/g minimization strategy. For small fish, the mortality risk of migrating far in the marine environment may carry an unfavorably high mortality risk, both because smaller fish are vulnerable to a wider range of predators and they have not accumulated sufficient energy reserves to survive the journey.

Calculating trade-offs that constrain the maximization of fitness is a core component of life history analysis (Stearns 1992; Roff 2002; Schaffer 2004) and the μ/g rule of Werner and Gilliam (1984) is a simple distillation of this concept. However, the simplicity of the rule diminishes its operational value. A pointed criticism of the μ/g rule was levied by Ludwig and Rowe (1990), who addressed the necessity for trade-off calculations to incorporate time constraints. They point out that while the μ/g rule assumes continuous reproduction over an indefinite time period, many organisms, including American shad, reproduce episodically and must attain some state by a given time for successful reproduction. By including this time constraint in their analytical model of the trade-offs between energy accumulation and mortality risk, Ludwig and Rowe (1990) conclude that an optimal foraging strategy depends on both the current

weight of the individual and the remaining time until a life history event such as reproduction. Their analysis leads to the conclusion that risky behaviors with greater payoffs are more likely in early stages, because small individuals have accumulated fewer resources and have less to lose, in a fitness sense, than larger ones. Conservative behaviors are then predicted for larger size classes. This increase in risk aversion with size was termed the 'asset-protection principle' by Clark (1994). On the face of it, an asset-protection strategy would seem to run counter to the hypothesis that American shad delay their long-distance migration until a certain size is attained, assuming that it is riskier to migrate along the coast. However, when Ludwig and Rowe (1990) analyze optimal strategies for switching between two habitats, beginning in one with low growth and low mortality rates and ending in one with high growth and high mortality, switching does occur after a minimum size is attained. This minimum size varies depending on the amount of elapsed time, accounting for variability in behavioral decisions within a population that contains individuals of different initial sizes. If these habitats were evaluated with a simple μ/g rule, the slow growth, low mortality habitat would be preferred indefinitely, but the addition of a time constraint induces a habitat switch once a minimum size is attained that reduces the mortality risk in the subsequent environment. Thus the asset-protection principle applies, but is constrained if the mortality risk in one habitat is too great to permit any foraging activity. Size, in the context of time constraints, theoretically mediates behavioral decisions to switch between habitats and allows movement to habitats that would have been intolerably risky at smaller sizes.

An alternative approach to analyzing behavioral decisions comes from the literature on terrestrial species and emphasizes the direct costs of migration. A simple

model of migration was put forth by Baker (1978), who considered the migratory cost M and the suitability of two habitats h_1 and h_2 , where suitability is dependent on measures of reproductive success due to residency in each habitat. In Baker's formulation, the decision to migrate from one habitat to another is favored when

$$(5.1) \quad h_1 < h_2 M$$

or the benefit of residency in habitat 2 outweighs the benefit of habitat 1, after accounting for the mortality risk of travel. The variables h and M are considered to vary with ontogeny and among individuals, and the initiation of migration can be either facultative or obligatory once the migration threshold is reached. This model is conceptually similar to the μ/g rule, with an individual assessing the relative trade-offs of remaining in one location or moving to another, and acting to maximize their fitness benefit. While Baker's model is simple and potentially comprehensive, a necessary drawback of simplicity is a limited ability to test the model empirically (Ketterson and Nolan 1983). Still, the model has general heuristic value when considering the forces driving migratory behavior.

In an exploration of the physiological costs of migration, Roff (1988) assessed bioenergetic constraints on life history traits in fishes and other migratory taxa. Body size was chosen as the primary variable of interest for this analysis since it correlates strongly with many life history traits including fecundity and development time. Energetic costs of movement were measured in terms of the energy that could be potentially converted to eggs, a kind of fecundity equivalence per distance traveled. Thus,

$$(5.2) \quad FED = \frac{NV}{C}$$

where FED is the fecundity equivalent distance in km, N is the number of calories in the gonads of a mature female fish, V is the migration speed that minimizes energy utilization, and C is the rate at which calories are expended at speed V (Roff 1988). C is assumed to be two times the standard metabolic rate, which scales with fish length (Ware 1978; Webb and Weihs 1983). This metabolic scaling also results in an increased aerobic capacity with mass (Killen et al. 2007). Hydrodynamically, the combined forces of drag, gravity, buoyancy, and thrust in a viscous fluid environment act such that larger fish expend less energy at a given speed than smaller ones (Weihs 1984, 1987). Thus size affects the speed of minimum energy expenditure, and Roff (1988) concluded that when converting stored gonadal energy into distance, a larger fish should in principle travel farther than a smaller fish. Bioenergetic models support the theory, then, that fish do not undertake long-distance migrations until they reach some minimum size where the journey is favored in energetic or fitness terms.

If models suggest ontogenetic shifts in migratory distance, is there empirical support for this hypothesis? Most investigations focus on the energetic requirements and migration distances of anadromous fishes in freshwater habitats, either during their juvenile downstream transitions or their upstream spawning migrations (see Hendry and Stearns 2004). Indeed, it has been explicitly shown for juvenile American shad that size and age determine the timing of migrations downriver (Limburg 1996a). Limburg (1996b) placed these freshwater migration strategies in a life history context using a dynamic programming model to assess the fitness consequences of habitat switching in the Hudson River. The model suggest juvenile American shad in the Hudson respond to the combined forces of predation risk, food availability, and adverse temperatures by

moving to habitats that optimize their fitness at a given size. Secor and Piccoli (1996) reported size-dependent emigration of female striped bass (*Morone saxatilis*) from fresh water through the estuarine gradient and hypothesized that this movement was the result of trade-offs between increased predation risks and foraging opportunities with increased salinity. Data on marine migratory patterns and distances traveled of fish in the marine environment are scarcer. However, several taxa appear to show correlations between marine migration distance and size. In evaluating his bioenergetic model, Roff (1988; 1991) compared migratory and non-migratory taxa and found migration to be more prevalent among species with larger adult sizes, and the same pattern was evident among gadiform species within the same order. Within a species, evidence also suggests size-based differences in marine migrations. A long-standing paradigm of subpopulation structuring in Pacific sardine (*Sardinops sagax*) comes from the observation that larger individuals range from California to the Gulf of Alaska while smaller fish are restricted to southern California (Clark 1935; Clark and Janssen 1945; Felin 1954; Smith 2005). Anadromous populations of brown trout (*Salmo trutta*) in the eastern Atlantic generally forage in shallow coastal waters, but long distance migrations far offshore have been observed in large individuals (Klemetsen et al. 2003). Atlantic cod (*Gadus morhua*) have often been observed to have an ontogenetic threshold to long-distance migratory behavior (Godø 1984; Rose 1993; Hanson 1996; Anderson and Gregory 2000). In a recent example, a mark-recapture study on a coastal population reported that smaller cod were recaptured close to tagging sites while the only cod recaptured at distances greater than 100 km were greater than 50 cm long (Lawson and Rose 2000). In a dramatic large-scale example of ontogenetic variation in migratory behavior, electronic archival and pop-up

satellite tags revealed that Atlantic bluefin tuna (*Thunnus thynnus*) do not undertake trans-oceanic migrations from the western to the eastern Atlantic until they reach lengths of 200 cm (Block et al. 2005). In summary, a number of taxa delay their long-distance migrations until they reach large sizes, and it is reasonable to hypothesize a similar strategy exists for American shad.

Theoretical and empirical evidence strongly supports the theory of ontogenetic shifts in migratory behavior, even after the transition to the marine environment. The marine phase of a diadromous life cycle can be hazardous, with survival rates for Atlantic salmon ranging from 30% to less than 5% (Hansen and Quinn 1998). This mortality has the potential to significantly restructure populations, both in terms of abundance and demography. Indeed, for American shad, marine mortality differed for individuals emigrating from the Hudson River throughout the season, with higher survival to recruitment for early and late emigrants than those emigrating at intermediate dates (Limburg 2001). The marine phase of the life cycle thus has the potential to act as a significant selective environment in which asset protection and fitness maximization are favored strategies, particularly for immature fish. This is not to suggest that decisions to commence long-distance migrations are fixed and one threshold migration size fits all. This decision is likely dynamic, dependent on environmental conditions, food availability, year class strength, and the presence of predators. Behavioral strategies are also likely to vary significantly among populations, since the migratory risk of traveling to the Bay of Fundy will differ substantially for fish originating from southern or northern regions. Moreover, density-dependence can play a significant role in structuring ontogenetic niche shifts (de Roos et al. 2002; 2003). Migration can minimize niche

overlap of successive cohorts, reducing intra-population competition for resources. In a possible scenario for American shad, a cohort that enters the estuarine or coastal marine environment may remain close to their natal river for approximately a year until the subsequent cohort begins to arrive. If the subsequent cohort is abundant enough to compete actively with the older fish, the older fish may commence their migration to distant, productive northern waters. Alternatively, low abundances may allow overlapping year classes to remain in the same coastal habitats without fitness consequences. All of these factors should play an integrated role in shaping the migratory strategy of American shad in the marine environment.

Because the possible contributing factors to behavioral decisions are numerous, it will likely be difficult to gather evidence in support of ontogenetic migratory shifts. High inter-annual variability in stock compositions could result from variability in the strength of each factor or simple stochasticity in population distributions that cannot be linked to identifiable variables. Investigating causative links among migratory behaviors, population dynamics, and abiotic processes may be prohibitively complex. However, the occurrence of ontogenetic shifts in migratory behavior in the marine environment can be documented using tools like otolith chemistry. Monitoring changes in the composition of a year class over time could be achieved by sampling American shad from Minas Basin in successive summers and then determining stock compositions based on geochemical signatures in otoliths. Increasing abundances of southern stocks and a diversification of the mixed stock composition with time would be necessary, although not sufficient, evidence supporting ontogenetic migratory shifts. This would represent a major step

forward in our understanding of migratory dynamics of American shad in the marine environment, a phase of their life history that we have only begun to explore.

Appendix 1

WATER, NOT FOOD, CONTRIBUTES THE MAJORITY OF STRONTIUM AND BARIUM DEPOSITED IN THE OTOLITHS OF A MARINE FISH

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ABSTRACT

We quantified the relative contributions of food and water to strontium (Sr) and barium (Ba) deposited in otoliths from juvenile mummichogs (*Fundulus heteroclitus*). Fish were reared in seawater spiked with ^{86}Sr and ^{137}Ba significantly beyond natural values to give food and water distinct isotopic signatures. Elemental abundances (Sr:Ca and Ba:Ca) and isotopic ratios ($^{88}\text{Sr}/^{86}\text{Sr}$ and $^{138}\text{Ba}/^{137}\text{Ba}$) were quantified in water samples using solution-based inductively coupled plasma-mass spectrometry (ICP-MS) and $^{88}\text{Sr}/^{86}\text{Sr}$ and $^{138}\text{Ba}/^{137}\text{Ba}$ ratios in otoliths were quantified using laser ablation ICP-MS. The relative contributions of food and water sources to otolith aragonite were assessed using a simple linear isotopic mixing model. Water sources contributed 83% of Sr and 98% of Ba in otoliths formed in spiked seawater. Our results indicate that water chemistry is the dominant factor controlling the uptake of Sr and Ba in the otoliths of marine fishes. As a result, chemical signatures recorded in otoliths of marine fishes should reflect the ambient water composition of these elements at the time of deposition.

A1.1 INTRODUCTION

For a century, fish otoliths (ear stones) have been used to study a wide array of topics in fish biology. Otoliths form by the periodic deposition of daily and annual increments, which allow researchers to determine ages and growth rates of individuals (Campana and Nielson 1985; Dwyer et al. 2003). More recently, geochemical analyses of otoliths, often targeting specific growth increments, have been used to reconstruct thermal histories (Patterson et al. 1993; Gao and Beamish 2003), identify fisheries management units (Edmonds et al. 1989; Rooker et al. 2003) and determine migration pathways (Limburg 1995; Secor 2001; Thorrold et al. 2001).

Several properties of otoliths make them useful and reliable tools. First, otoliths grow throughout the life of a fish by the addition of successive layers of aragonitic calcium carbonate (Campana 1999). These layers are often manifested as daily or yearly increments, and therefore otoliths may act as biological chronometers recording the age and growth rates of individual fish. Second, otoliths are acellular and metabolically inert and thus are not subject to resorption after the calcareous material accretes (Campana and Nielson 1985). As a result, the chemical composition of a given layer remains constant in time. These properties allow the environmental history of a fish to be reconstructed by analyzing sections of an otolith corresponding to the time periods of interest.

Although studies involving otolith geochemistry are increasingly prevalent (Campana and Thorrold 2001), few investigators have examined the sources of material deposited in otolith aragonite. Cations enter the blood plasma either through intestinal digestion or by transport across the branchial membranes (Campana 1999). These ions

travel via the bloodstream to the inner ear and then across a membrane into the endolymph, where they are available for crystallization. Otolith composition will, therefore, reflect the relative contribution of water and dietary sources to the ions that ultimately precipitate out of solution at the depositional surface of the otolith. The distinction between water and diet is important because we would only expect otolith chemistry to reflect dissolved concentrations in the environment if water were the primary source of ions in the otoliths.

Traditionally, water was thought to contribute the most to the composition of calcareous structures in teleosts (Schiffman 1961; Berg 1968; Simkiss 1974; Hoff and Fuiman 1995; Farrell and Campana 1996; Gallahar and Kingsford 1996). However, dietary composition may also influence otolith chemistry. The elemental composition of otoliths has been shown to reflect diet in both freshwater (Limburg 1995; Kennedy et al. 2000) and marine fishes (Buckel et al. 2004). We sought to address these discrepancies by rearing juvenile mummichogs (*Fundulus heteroclitus*) in seawater spiked with enriched Sr and Ba isotopes beyond natural isotopic ratios found in food. This experimental approach provided a definitive test of the relative contributions of water and diet to otolith composition.

A1.2 MATERIALS AND METHODS

A1.2.1 Fish rearing conditions

Mummichogs are estuarine killifish that occupy marsh habitats along the East Coast of North America from Florida to Newfoundland (Collette and Klein-MacPhee

2002). Individuals were collected from Eel Pond in Woods Hole, Massachusetts using baited minnow traps set during spring tides of June 2002. Eggs were manually extracted from gravid females and fertilized in vitro by commingling eggs and diced testes from sacrificed males in Petri dishes containing filtered natural seawater. Testes pieces were removed after 24 hours. Eggs developed in Petri dishes at a room temperature of 19°C, with seawater changed every three days. Eggs hatched after approximately 28 days and larvae were then transferred to experimental tanks.

High-density polyethylene tanks were filled with 10 L filtered natural seawater at a salinity of 30‰. Three replicate control tanks contained unaltered seawater while three treatment tanks were spiked with 1 mg ^{86}Sr and 50 μg ^{137}Ba each. Stable isotopes were purchased from Oak Ridge National Laboratory, Tennessee, USA. After treatment tanks were spiked, ten fish were added to each tank, except one control tank that contained only nine. Fish were fed daily on TetraColor™ Tropical Flakes for the first 28 days and after that twice daily until harvest. Tanks were covered to minimize evaporation and maintained in a constant room temperature of 19°C under a 14-h light/10-h dark cycle. Despite a constant internal filtration system, food accumulated on the bottom of the tanks. To maintain water quality, we changed 1/3 of the water volume weekly after 26 days of the experiment. Water added to treatment tanks was spiked with ^{86}Sr and ^{137}Ba to maintain constant treatment ratios throughout the experiment. Fish were harvested and frozen after 70 days. High mortality rates in three of the tanks meant that we were only able to harvest fish from three treatment tanks and one control tank (Table A.1).

A1.2.2 Water sampling

Water samples were taken weekly from each tank with acid-washed polypropylene syringes. Samples were processed in a class 100 clean room where 10 ml of water was filtered through 0.2 μm acid-washed polypropylene filters, acidified with 200 μl concentrated ultrapure HNO_3 , and then refrigerated at 5°C. Immediately before analysis samples were returned to room temperature, diluted 50:1 with 2% ultrapure HNO_3 and spiked with ^{115}In at 1 ng.g^{-1} for use as an internal standard.

All samples were run on a Finnigan MAT Element ICP-MS using a self-aspirating 50 l.min^{-1} PFA nebulizer attached to a quartz cyclonic spray chamber. Concentrations of Ca, Sr and Ba were quantified by monitoring ^{43}Ca , ^{88}Sr and ^{138}Ba in low resolution, and then standardizing blank-corrected isotope intensities to ^{115}In following the method of Field et al. (1999). Elemental concentrations were expressed as molar ratios to Ca because Sr and Ba in otoliths vary as a function of element:Ca ratios rather than absolute concentration (Bath et al. 2000; Kraus and Secor 2004). Estimates of analytical precision (relative standard deviation) of a reference seawater run at intervals throughout the analyses ($n = 4$) were 3.5% for Sr:Ca and 4.5% for Ba:Ca.

Isotopic ratios of Sr and Ba were measured in the water samples using the same sample introduction system and diluted solutions that were assayed for elemental concentrations. We measured ^{86}Sr , ^{88}Sr , ^{137}Ba and ^{138}Ba in water samples, the seawater standard, and 2% HNO_3 blanks. Isotopic ratios ($^{88}\text{Sr}/^{86}\text{Sr}$ and $^{138}\text{Ba}/^{137}\text{Ba}$) were then calculated from blank-corrected abundances following correction for instrument mass

bias assuming natural isotopic ratios ($^{88}\text{Sr}/^{86}\text{Sr} = 8.375$ and $^{138}\text{Ba}/^{137}\text{Ba} = 6.385$) were present in the seawater standard.

A1.2.3 Otolith chemistry

Sagittal otolith pairs were removed from each fish and cleaned of tissue. One otolith from each pair was randomly selected and mounted on a petrographic slide with cyanoacrylic glue. Mounted otoliths were ground to the midplane on 3 μm lapping film. Otoliths were immersed in ultrapure Milli-Q water, left to soak for 24 h, and then sonicated for 2 minutes to remove surface contamination. After a triple-rinse, otoliths were left to dry in a class 100 laminar flow hood for 24 h. Once dry, otoliths were remounted on slides using double-sided tape for Sr and Ba isotopic analyses.

Isotopic ratios of Sr and Ba in each otolith were quantified with a New Wave Research UP213 laser ablation system coupled to a Thermo Finnigan Element ICP-MS. Instrument set-up was similar to that outlined by Günther and Heinrich (1999) as modified by FitzGerald et al. (2004). Briefly, a He gas stream was used to carry ablated material from the laser cell to the ICP-MS. The carrier gas was then mixed with the Ar sample gas and a wet aerosol (2% HNO_3) in the concentric region of the quartz dual inlet spray chamber. The wet aerosol was supplied by a self-aspirating PFA micro-flow (20 $\text{m}\cdot\text{min}^{-1}$) nebulizer attached to a CETAC ASX100 autosampler. The laser software was used to trace out a 150x150 μm raster near the edge of the otoliths that consisted of material laid down during the experimental period. Blank-corrected isotopic abundances were corrected for instrument mass bias using an otolith reference material (Yoshinaga

2000) that was dissolved in 2% HNO₃ and diluted to a final Ca concentration of 40 g.g⁻¹. As with the seawater standard, we assumed that the otolith reference material contained natural isotopic ratios for both ⁸⁸Sr/⁸⁶Sr and ¹³⁸Ba/¹³⁷Ba. Precision of the technique was assessed by assaying ⁸⁸Sr/⁸⁶Sr and ¹³⁸Ba/¹³⁷Ba ratios in the aragonitic skeleton of a marine sclerosponge at regular intervals throughout the otolith analyses. Calculated values from the sclerosponge (⁸⁸Sr/⁸⁶Sr = 8.33 ± 0.1 [1 SD], ¹³⁸Ba/¹³⁷Ba = 6.43 ± 0.03 [1 SD], n = 6) were within 2 SD of natural values for the Sr and Ba isotopic ratios.

Finally, the percent contribution of the water to Sr and Ba deposited in the otoliths during the experiment was calculated with a mixing model following Kennedy et al. (2000):

$$(A1.1) \quad \%element_{(from\ water)} = \left(1 - \left(\frac{isotope\ ratio_{water} - isotope\ ratio_{otolith}}{isotope\ ratio_{water} - isotope\ ratio_{natural}} \right) \right) \times 100$$

Isotopic ratios for the water used in this model were an average of samples taken from each spiked tank over the course of the experiment. This calculation assumed that the food contains natural isotopic ratios (⁸⁸Sr/⁸⁶Sr = 8.375 and ¹³⁸Ba/¹³⁷Ba = 6.385) because these ratios are invariant in nature (Lide 1995). The contribution of food to otolith aragonite was then calculated as

$$(A1.2) \quad \%element_{(from\ food)} = 100 - (\%element_{(from\ water)})$$

A1.3 RESULTS

A1.3.1 Water chemistry

Elemental concentrations within the rearing tanks varied between 8.5 to 9.3 mmol.mol⁻¹, for Sr:Ca, and between 10 and 37 mol.mol⁻¹ for Ba:Ca (Figure A1.1). These changes presumably reflected chemistry differences in the seawater line from which replacement water was taken during the experiment. Isotopic ratios within the tanks during the experiment were, however, more constant (Figure A1.1). The three spiked tanks had ⁸⁸Sr/⁸⁶Sr ratios of 6.64 ± 0.09, 6.62 ± 0.09, and 6.7 ± 0.2 (SD), and ¹³⁸Ba/¹³⁷Ba ratios of 1.6 ± 0.3, 2.1 ± 0.2, and 2.4 ± 0.2 (SD). As expected, there was little variation in isotopic ratios within the control tank during the experiment, with mean values for both ⁸⁸Sr/⁸⁶Sr (8.376 ± 0.007) and ¹³⁸Ba/¹³⁷Ba (6.359 ± 0.014) being within 2 SD of natural ratios. Although fish mortality precluded the inclusion of multiple control tanks, little variability among control tanks in isotopic ratios would be expected since natural ratios are constant (Lide 1995).

A1.3.2 Isotopic ratios in otoliths

Both ⁸⁸Sr/⁸⁶Sr and ¹³⁸Ba/¹³⁷Ba ratios in otoliths of fish from the control tank were within 2 SD of natural ratios of these isotopes in the environment (Table A1.1). However, isotopic ratios of otoliths from fish from the treatment tanks were significantly more enriched in both ⁸⁶Sr and ¹³⁷Ba over natural levels, indicating a significant contribution of water to otolith chemistry. We calculated the percent contributions of water and diet to Sr and Ba in the otoliths using a simple mixing model between the natural ratios of ⁸⁸Sr/⁸⁶Sr and ¹³⁸Ba/¹³⁷Ba found in the diet and the altered ratios measured in each of the

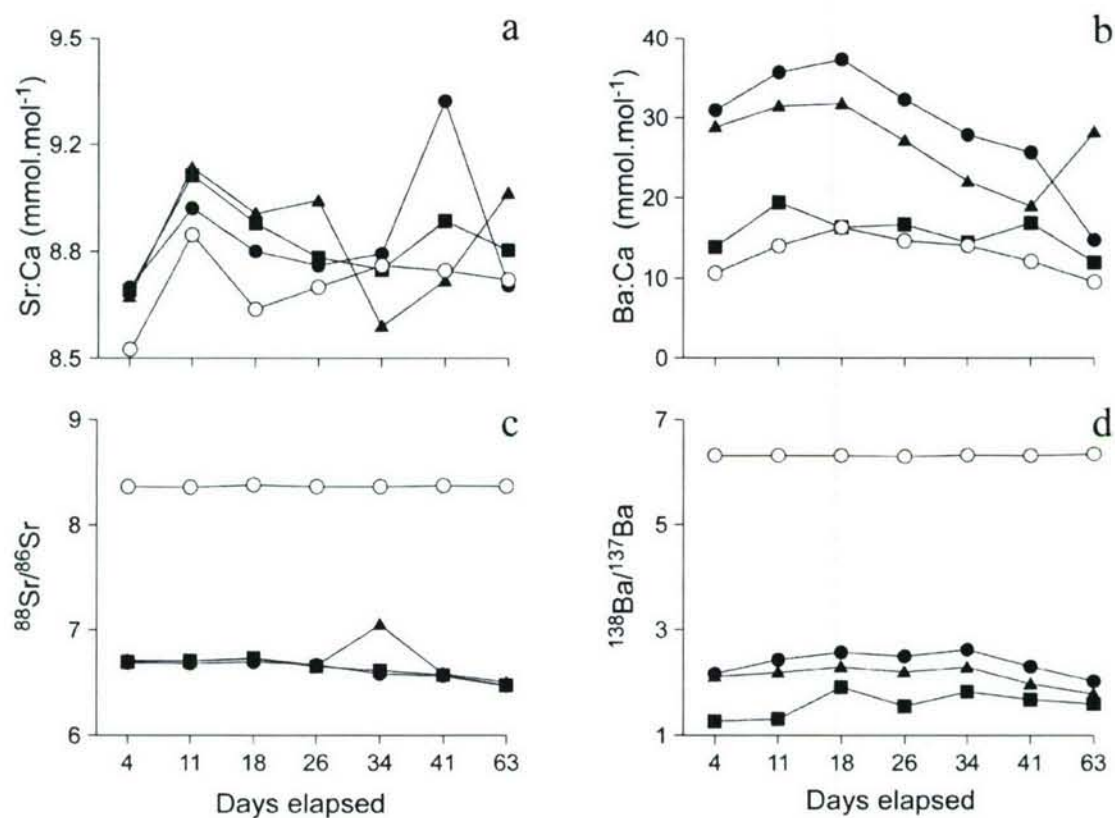


Figure A1.1 Elemental ratios (Sr:Ca and Ba:Ca; a,b) and isotopic ratios (⁸⁸Sr/⁸⁶Sr and ¹³⁸Ba/¹³⁷Ba; c,d) in water samples taken over the course of the experiment from three treatment tanks spiked with enriched ⁸⁶Sr and ¹³⁷Ba (solid symbols) and the control tank with natural isotopic ratios (open symbols).

Table A1.1 Isotopic ratios (\pm SD) in water (H₂O) and otoliths (OTO) from rearing tanks containing seawater spiked with ⁸⁶Sr and ¹³⁷Ba (Tanks 1, 2 and 3) and the control tank. Water values are averages of 7 samples taken over the course of the experiment; *n* represents the number of surviving fish in each tank used for otolith analyses. The percent contribution of water (%H₂O) in otoliths of fish from each spiked tank was calculated from a linear mixing model.

		Sr			Ba		
	<i>n</i>	[^{88/86} Sr]H ₂ O	[^{88/86} Sr]OTO	%H ₂ O	[^{137/138} Ba]H ₂ O	[^{137/138} Ba]OTO	%H ₂ O
Tank 1	1	6.64 \pm 0.09	7.06	76	1.59 \pm 0.25	1.55	101
Tank 2	3	6.62 \pm 0.09	6.9 \pm 0.3	86	2.38 \pm 0.22	2.55 \pm 0.01	96
Tank 3	9	6.71 \pm 0.17	6.9 \pm 0.2	86	2.12 \pm 0.19	2.29 \pm 0.15	96
Control	4	8.38 \pm 0.01	8.3 \pm 0.1	-	6.36 \pm 0.01	6.42 \pm 0.02	-

rearing tanks (Table A1.1). Based on the model, water contributed $83\% \pm 6$ (SD) to Sr and $98\% \pm 3$ (SD) to Ba in the otoliths of the experimental fish, averaged over treatment tanks. Consequently, food only contributed approximately 17% of Sr and 2% of Ba to otolith aragonite.

A1.4 DISCUSSION

Our study used a novel technique to investigate sources of two alkali earth metals (Sr and Ba) incorporated into the aragonitic otoliths of a marine fish. We reared fish in seawater that was spiked with stable isotopes of Sr and Ba beyond any natural variation in the environment. The presence of unique isotopic signatures in the water allowed us to use a simple mixing model to determine the relative contributions of the food and water to the Sr and Ba ions deposited in otoliths of the larval fish during the experiment. Isotopic ratios of food sources were not directly measured in this experiment but the food sources must have contained natural isotopic ratios of both $^{88}\text{Sr}/^{86}\text{Sr}$ and $^{138}\text{Ba}/^{137}\text{Ba}$ because these ratios are invariant in nature (Lide 1995). The results demonstrated that Sr and Ba in the otoliths were derived primarily from the ambient water. Over 80% of otolith Sr and more than 95% of otolith Ba originated from the water in which the fish were reared.

The result that water contributed most of the Sr and Ba ions ultimately deposited in otoliths is significant for studies that attempt to relate otolith chemistry to that of the ambient environment experienced by individual fish (Campana 1999). If food contributed the majority of otolith Sr and Ba, otolith composition would likely be decoupled from element concentrations in the surrounding water. However, our results

suggest that Sr and Ba in otoliths are likely to be sensitive to changes in ambient levels in the environment. Indeed, these data complement a growing number of studies that have documented significant correlations between otolith composition and the concentrations of several elements in ambient waters (Bath et al. 2000; Milton and Chenery 2001; Elsdon and Gillanders 2002).

Previous workers have produced conflicting results concerning the relative importance of diet to otolith elemental composition (e.g., Farrell and Campana 1996 and Kennedy et al. 2000). The research may be usefully divided into those that have manipulated the elemental composition of diets and studies that have used isotopic variations to quantify the relative contribution of water and diet to otolith composition. In those studies that altered dietary intake of elements, several authors reported no influence of diet (Hoff and Fuiman 1995; Milton and Chenery 2001), while others found detectable effects of diet on otolith chemistry (Limburg 1995; Gallahar and Kingsford 1996; Buckel et al. 2004). Of the studies that reported detectable dietary effects on otolith chemistry, Limburg (1995) and Gallahar and Kingsford (1996) found small effects on otolith Sr content when fish were fed Sr-spiked food. Buckel et al (2004), however, reported that Sr increased by approximately 20%, and Ba increased by 100%, in otoliths of juvenile bluefish fed prey that differed in Sr and Ba concentration by approximately 250%. Unfortunately, Buckel et al. (2004) measured neither water chemistry nor the Ca concentration of the prey items in their experiment. Nonetheless, assuming that Ca concentrations were similar in both prey types, we calculated that water likely contributed approximately 70% of the Sr and 40% of the Ba deposited in the otoliths of juvenile bluefish from the data reported by Buckel and co-workers. Clearly, more

experimentation is needed to ascertain if the differences between the results reported here and those of Buckel et al. (2004) are due to inter-specific variability, salinity effects (juvenile bluefish were reared at ~23‰, juvenile mummichogs at 30‰), or some artifact of the one of the experimental approaches.

Researchers using isotopic techniques to examine dietary effects on otolith chemistry have addressed the issue by directly measuring the relative contribution of food and water to the ions that are ultimately deposited in otoliths. Farrell and Campana (1996) quantified the relative contributions of food and water to otolith composition using radio-labeled Ca and Sr isotopes and found 75% of Ca and 88% of Sr in otoliths of freshwater Nile tilapia derived from the water. In contrast, Kennedy et al. (2000) reported 70% of Sr in otoliths of Atlantic salmon reared in freshwater environments originated from dietary sources based on natural isotopic variations in water and food. While a conclusion about the relative contributions of food and water to otoliths of freshwater fish remains ambiguous, we used isotopic techniques to demonstrate that marine fish depend primarily on water sources for Sr and Ba accreted on otoliths.

If fishes rely on ambient water as their primary source for otolith material, then Sr and Ba isotopic ratios in the otolith should reflect those of the water column. Both Sr and Ba substitute easily for Ca ions in the aragonitic matrix because they exhibit ionic radii similar to Ca (Speer 1983). In addition, they are non-essential elements for fishes and undergo less direct regulation at interfaces along the pathway to accretion compared to essential elements such as Na and Ca (Campana 1999). The relative importance of dietary and water sources to the otoliths may change depending on Ca availability in the water column (Berg 1968; Farrell and Campana 1996). Because Ca concentration varies

with salinity, freshwater fishes may compensate for decreased water Ca by deriving relatively more ions from their food. In order to fully understand variations in the importance of food and water sources to otolith formation, experiments testing the link between salinity and dietary contribution are needed to determine whether freshwater fishes rely more on food sources for otolith material than do marine fishes. However, our evidence shows that marine fishes do indeed draw primarily upon water sources for non-essential minor and trace elements incorporated into otoliths.

ACKNOWLEDGEMENTS

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APPENDIX 2

ANALYTICAL DATA FOR SAMPLES USED IN CHAPTER 2

Table A2.1 Juvenile American shad collected from rivers in 2000, 2001, and 2002 and used for analyses reported in Chapter 2. Each row reports corrected elemental and isotopic ratios obtained from the otoliths of one individual fish. River codes: Exe: Exeter River, NH. Con: Connecticut River, CT. Hud: Hudson River, NY. Del: Delaware River, NJ. Sus: Susquehanna River, MD. UpC: Upper Chesapeake Bay, MD. Pot: Potomac River, MD. Rap: Rappahannock River, VA. Mat: Mattaponi River, VA. Pam: Pamunkey River, VA. StC: Santee-Cooper River, SC. Alt: Altamaha River, GA.

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹³ C VPDB	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Exe	2001	0.132	5.63	0.471	4.06	-18.34	-9.07	0.71706
Exe	2001	0.097	6.46	0.444	4.89	-16.38	-8.06	0.71704
Exe	2001	0.113	4.81	0.506	4.03	-16.96	-8.10	0.71710
Exe	2001	0.228	6.30	0.465	4.62	-17.43	-8.28	0.71709
Exe	2001	0.104	7.18	0.482	5.47	-18.28	-8.16	0.71701
Exe	2001	0.128	4.00	0.469	6.82	-16.91	-8.05	0.71698
Exe	2001	0.209	4.72	0.474	3.95	-17.38	-8.10	0.71703
Exe	2001	0.245	3.87	0.474	6.93	-17.68	-8.21	0.70947
Exe	2001	0.111	6.85	0.450	7.85	-17.76	-8.23	0.71700
Exe	2001	0.108	6.88	0.467	6.61	-16.38	-7.92	0.71709
Exe	2001	0.092	7.55	0.476	7.12	-17.48	-7.98	0.71696
Exe	2001	0.159	6.85	0.452	4.09	-18.09	-8.46	0.71713
Exe	2001	0.078	4.56	0.449	5.86	-18.12	-8.33	0.71696
Exe	2001	0.216	5.08	0.495	5.19	-18.18	-8.27	0.71709
Exe	2001	0.095	5.23	0.414	4.91	-16.51	-7.90	0.71690
Exe	2001	0.070	5.97	0.500	9.21	-17.37	-8.37	0.71701
Exe	2001	0.127	5.15	0.453	4.01	-17.22	-8.09	0.71707
Exe	2001	0.111	7.10	0.502	8.28	-16.76	-8.00	0.71690
Exe	2001	0.080	5.63	0.449	5.86	-17.07	-7.74	0.71691
Exe	2001	0.123	4.86	0.476	5.05	-17.25	-8.14	0.71700
Exe	2001	0.152	5.13	0.475	4.03	-16.28	-7.99	0.71703
Exe	2001	0.131	5.75	0.463	4.07	-15.82	-7.78	0.71715

Table A2.1 continued

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{13}\text{C}$ VPDB	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
Exe	2001	0.119	7.40	0.455	6.11	-17.15	-7.93	0.71706
Exe	2001	0.089	5.46	0.472	6.16	-15.64	-7.67	0.71693
Exe	2001	0.141	3.96	0.470	4.67	-17.24	-8.25	0.71702
Exe	2001	0.198	5.47	0.538	9.09	-17.31	-8.03	0.71707
Exe	2001	0.129	5.69	0.497	4.38	-17.77	-8.47	0.71710
Exe	2001	0.164	4.09	0.436	4.97	-17.02	-7.83	0.71696
Con	2001	0.075	2.13	0.529	8.92	-14.43	-11.37	0.71335
Con	2001	0.102	3.80	0.476	8.65	-14.52	-11.22	0.71347
Con	2001	0.103	2.88	0.430	6.33	-15.16	-11.13	0.71337
Con	2001	0.152	3.51	0.526	8.12	-15.64	-11.13	0.71340
Con	2001	0.090	4.50	0.505	9.01	-15.11	-10.97	0.71348
Con	2001	0.132	3.25	0.602	9.95	-14.84	-11.19	0.71339
Con	2001	0.131	3.18	0.548	9.91	-15.07	-11.26	0.71327
Con	2001	0.113	3.52	0.602	11.63	-13.13	-11.22	0.71298
Con	2001	0.078	3.84	0.512	9.30	-13.14	-11.35	0.71307
Con	2001	0.097	3.74	0.584	9.39	-13.18	-10.99	0.71308
Con	2001	0.106	3.47	0.467	10.07	-13.28	-10.85	0.71308
Con	2001	0.088	3.37	0.655	11.42	-13.49	-11.49	0.71310
Con	2001	0.075	3.27	0.630	17.02	-13.03	-11.34	0.71301
Con	2001	0.072	3.68	0.532	10.56	-13.82	-11.15	0.71348
Con	2001	0.100	4.01	0.659	13.66	-14.48	-11.27	0.71345
Con	2001	0.078	3.52	0.661	15.80	-12.87	-11.04	0.71327
Con	2001	0.097	3.26	0.585	13.40	-13.17	-11.16	0.71290
Con	2001	0.084	4.94	0.480	9.13	-13.60	-10.89	0.71305
Con	2001	0.091	3.03	0.525	10.78	-12.71	-11.30	0.71313
Con	2001	0.065	3.35	0.585	14.63	-14.05	-11.17	0.71394
Con	2001	0.090	3.64	0.457	9.75	-13.67	-12.01	0.71308
Con	2001	0.092	5.18	0.506	9.50	-13.66	-12.05	0.71315
Con	2001	0.104	4.28	0.457	9.47	-13.83	-12.16	0.71303
Con	2001	0.122	3.96	0.481	13.06	-14.67	-11.82	0.71338
Con	2001	0.144	3.46	0.502	7.32	-14.09	-11.96	0.71328

Table A2.1 continued

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹³ C VPDB	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Con	2001	0.069	3.34	0.441	8.90	-13.97	-12.07	0.71299
Con	2001	0.093	2.84	0.480	9.45	-13.75	-11.97	0.71305
Con	2001	0.093	3.21	0.467	9.09	-13.73	-12.09	0.71308
Hud	2000	0.097	2.45	0.414	5.72	-15.94	-10.25	0.71145
Hud	2000	0.085	3.76	0.427	6.21	-15.91	-10.49	0.71131
Hud	2000	0.082	2.40	0.441	6.04	-16.05	-10.56	0.71168
Hud	2000	0.057	2.67	0.444	6.90	-15.20	-10.21	0.71102
Hud	2000	0.039	1.49	0.482	7.58	-14.82	-10.29	0.71062
Hud	2000	0.081	4.21	0.451	6.39	-15.65	-10.38	0.71138
Hud	2000	0.087	3.05	0.479	6.30	-16.01	-10.54	0.71151
Hud	2000	0.075	2.84	0.406	5.36	-16.10	-10.34	0.71172
Hud	2000	0.064	1.67	0.442	5.38	-15.47	-10.34	0.71064
Hud	2000	0.107	2.84	0.444	6.10	-14.28	-10.11	0.71060
Hud	2000	0.102	1.81	0.500	8.20	-15.05	-10.08	0.71072
Hud	2000	0.116	2.89	0.429	7.65	-15.80	-10.65	0.71090
Hud	2000	0.199	2.45	0.416	6.90	-15.91	-10.57	0.71120
Hud	2000	0.102	1.47	0.364	3.86	-15.97	-10.58	0.71179
Hud	2000	0.147	2.15	0.370	5.15	-15.77	-10.47	0.71107
Hud	2000	0.213	2.62	0.514	6.97	-15.65	-10.62	0.71148
Hud	2000	0.055	1.83	0.474	4.61	-14.46	-10.29	0.71061
Hud	2000	0.113	2.23	0.464	7.08	-15.18	-10.33	0.71077
Hud	2000	0.040	1.51	0.516	6.97	-15.29	-10.30	0.71256
Hud	2000	0.086	2.18	0.424	5.89	-15.08	-10.37	0.71053
Hud	2000	0.114	2.72	0.450	7.08	-14.81	-10.26	0.71064
Hud	2000	0.107	2.72	0.466	7.48	-15.10	-10.38	0.71052
Hud	2000	0.099	2.93	0.366	5.39	-16.71	-10.27	0.71114
Hud	2000	0.075	4.98	0.455	8.17	-15.85	-10.43	0.71576
Hud	2000	0.084	2.37	0.402	5.33	-15.52	-10.25	0.71203
Hud	2000	0.071	4.12	0.434	7.28	-15.67	-10.25	0.71138
Hud	2000	0.077	2.42	0.485	7.67	-14.97	-10.32	0.71087
Hud	2001	0.261	2.32	0.421	4.72	-15.74	-11.53	0.71101

Table A2.1 continued

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹³ C VPDB	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Hud	2001	0.181	1.41	0.472	4.57	-14.44	-11.11	0.71041
Hud	2001	0.081	2.02	0.438	4.27	-14.58	-11.02	0.71105
Hud	2001	0.096	2.42	0.457	4.86	-15.66	-11.10	0.71093
Hud	2001	0.065	1.93	0.460	4.57	-15.34	-11.51	0.71084
Hud	2001	0.261	3.00	0.420	4.02	-15.32	-11.15	0.71121
Hud	2001	0.094	2.44	0.475	5.59	-15.70	-11.20	0.71058
Hud	2001	0.097	2.54	0.464	3.69	-14.93	-11.02	0.71051
Hud	2001	0.106	2.27	0.451	4.77	-15.54	-11.60	0.71112
Hud	2001	0.147	3.36	0.468	5.67	-15.38	-11.48	0.71120
Hud	2001	0.125	1.91	0.473	5.04	-14.91	-11.30	0.71077
Hud	2001	0.172	2.48	0.363	3.83	-15.43	-11.61	0.71066
Hud	2001	0.197	2.54	0.357	4.24	-14.89	-10.99	0.71094
Hud	2001	0.111	2.53	0.459	5.14	-15.09	-11.25	0.71067
Hud	2001	0.075	2.44	0.473	4.80	-14.83	-10.86	0.71048
Hud	2001	0.084	3.05	0.565	6.80	-14.36	-11.22	0.71047
Hud	2001	0.155	1.83	0.445	4.95	-14.42	-11.34	0.71068
Hud	2001	0.228	3.15	0.456	5.96	-14.96	-11.37	0.71075
Hud	2001	0.160	2.16	0.469	4.10	-14.35	-11.22	0.71064
Hud	2001	0.187	1.69	0.481	5.01	-14.85	-11.38	0.71049
Hud	2001	0.227	2.15	0.451	4.39	-14.53	-11.33	0.71063
Hud	2001	0.094	1.62	0.440	5.26	-14.33	-11.15	0.71067
Hud	2001	0.085	2.39	0.533	8.70	-14.03	-11.32	0.71046
Hud	2001	0.139	2.61	0.556	6.10	-14.95	-11.12	0.71057
Hud	2001	0.110	2.17	0.471	5.45	-15.12	-11.53	0.71092
Hud	2001	0.100	3.32	0.482	4.38	-15.23	-11.58	0.71105
Hud	2001	0.126	1.90	0.456	4.70	-14.65	-11.04	0.71073
Hud	2001	0.130	2.04	0.512	5.79	-14.13	-11.07	0.71056
Del	2000	0.077	2.68	0.391	7.84	-14.36	-9.13	0.71238
Del	2000	0.076	2.40	0.409	8.66	-15.00	-9.24	0.71230
Del	2000	0.098	2.87	0.367	7.24	-14.53	-9.31	0.71234
Del	2000	0.104	2.17	0.380	8.82	-14.50	-9.30	0.71211

Table A2.1 continued

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{13}\text{C}$ VPDB	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
Del	2000	0.154	2.57	0.277	6.88	-14.76	-9.39	0.71229
Del	2000	0.084	1.81	0.367	9.99	-14.66	-9.38	0.71247
Del	2000	0.076	2.95	0.368	7.82	-14.86	-9.47	0.71334
Del	2000	0.062	2.17	0.378	6.89	-14.78	-9.41	0.71228
Del	2000	0.088	1.73	0.382	6.43	-14.76	-9.43	0.71241
Del	2000	0.067	2.48	0.394	6.07	-14.67	-9.00	0.71244
Del	2000	0.151	2.46	0.345	6.21	-14.73	-9.05	0.71243
Del	2000	0.125	2.70	0.401	6.75	-15.14	-9.94	0.71323
Del	2000	0.095	1.98	0.325	6.81	-13.76	-9.01	0.71240
Del	2000	0.185	2.26	0.374	6.37	-14.36	-9.24	0.71246
Del	2000	0.229	2.46	0.335	6.32	-13.84	-9.17	0.71262
Del	2000	0.144	1.96	0.401	7.37	-14.67	-9.55	0.71233
Del	2000	0.096	2.00	0.355	7.61	-14.41	-9.20	0.71250
Del	2000	0.396	1.35	0.297	10.46	-14.60	-8.78	0.71385
Del	2000	0.094	2.04	0.305	9.53	-12.69	-9.44	0.71257
Del	2000	0.104	4.84	0.329	16.18	-12.40	-9.61	0.71254
Del	2000	0.166	1.71	0.276	8.07	-14.61	-9.60	0.71260
Sus	2000	0.066	3.78	0.194	9.41	-15.22	-10.76	0.71223
Sus	2000	0.067	7.21	0.254	7.25	-14.06	-10.20	0.71281
Sus	2000	0.063	4.00	0.197	9.50	-14.80	-10.29	0.71206
Sus	2000	0.072	3.89	0.204	7.90	-15.22	-10.64	0.71299
Sus	2000	0.077	3.87	0.688	10.10	-13.57	-10.45	0.71041
Sus	2000	0.062	4.36	0.236	11.43	-15.01	-10.46	0.71265
Sus	2000	0.092	5.57	0.271	9.46	-14.21	-10.21	0.71302
Sus	2000	0.069	2.87	0.192	10.27	-13.72	-10.11	0.71265
Sus	2000	0.058	2.34	0.235	5.78	-13.65	-10.30	0.71265
Sus	2000	0.068	5.89	0.260	7.24	-13.20	-9.93	0.71286
Sus	2000	0.070	2.65	0.186	7.00	-14.79	-10.59	0.71211
Sus	2000	0.059	2.37	0.199	12.18	-15.46	-10.86	0.71224
Sus	2000	0.083	3.27	0.191	11.55	-14.22	-10.12	0.71202
Sus	2000	0.070	2.79	0.332	4.88	-14.27	-10.39	0.71306

Table A2.1 continued

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{13}\text{C}$ VPDB	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
Sus	2000	0.069	4.38	0.207	15.11	-14.88	-10.82	0.71224
Sus	2000	0.074	2.74	0.468	6.08	-12.19	-9.68	0.71064
Sus	2000	0.057	4.38	0.244	6.93	-13.08	-9.88	0.71306
Sus	2000	0.058	2.70	0.236	10.33	-14.10	-10.45	0.71258
Sus	2000	0.062	4.52	0.315	12.37	-14.83	-10.44	0.71300
Sus	2000	0.055	1.98	0.189	10.42	-15.42	-10.59	0.71194
UpC	2000	0.140	2.40	0.798	3.18	-13.70	-9.62	0.71049
UpC	2000	0.305	2.55	0.675	2.73	-12.49	-9.55	0.71055
UpC	2000	0.685	2.95	0.454	5.72	-14.27	-9.98	0.71165
UpC	2000	0.096	3.45	0.617	2.49	-13.01	-9.79	0.71053
UpC	2000	0.316	2.81	0.673	4.47	-13.04	-9.60	0.71088
UpC	2000	0.607	3.04	0.556	6.12	-13.07	-10.13	0.71131
UpC	2000	0.153	2.44	0.685	2.65	-12.62	-9.72	0.71062
UpC	2000	0.343	3.05	0.639	4.05	-12.99	-9.85	0.71058
UpC	2000	0.193	6.34	0.741	3.13	-14.41	-9.40	0.71049
UpC	2000	0.109	2.42	0.807	3.14	-12.42	-9.58	0.71074
UpC	2000	0.282	2.91	0.639	3.37	-13.04	-9.88	0.71055
UpC	2000	0.093	2.62	0.871	4.44	-12.02	-9.73	0.71109
UpC	2000	0.264	3.54	0.815	3.49	-12.64	-9.69	0.71104
UpC	2000	0.362	2.81	0.522	3.21	-13.07	-10.10	0.71091
UpC	2000	0.262	2.39	0.589	3.57	-13.17	-9.54	0.71088
UpC	2000	0.233	3.07	0.622	5.43	-12.32	-10.00	0.71133
UpC	2000	0.188	3.09	0.710	3.15	-13.75	-9.75	0.71067
UpC	2000	0.254	2.22	0.645	4.02	-13.66	-9.82	0.71056
UpC	2000	0.541	1.50	0.655	6.28	-12.90	-9.72	0.71061
UpC	2000	0.206	3.92	0.642	3.27	-13.72	-9.77	0.71060
UpC	2000	0.170	2.25	0.697	4.87	-12.88	-9.59	0.71120
UpC	2000	0.270	3.49	0.844	4.71	-13.05	-9.58	0.71083
UpC	2000	0.265	2.88	0.665	3.06	-12.22	-9.56	0.71060
UpC	2000	0.373	4.22	0.350	8.98	-14.14	-10.31	0.71181
UpC	2000	0.256	2.67	0.705	3.49	-12.58	-9.73	0.71042

Table A2.1 continued

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹³ C VPDB	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
UpC	2000	0.238	2.64	0.495	2.78	-14.40	-9.83	0.71068
UpC	2000	0.111	2.67	0.677	2.77	-13.30	-9.22	0.71080
UpC	2000	0.107	2.50	0.912	3.33	-13.00	-10.35	0.71088
UpC	2000	0.132	2.34	0.432	4.58	-12.35	-9.86	0.71166
Pot	2000	0.087	2.20	0.575	4.05	-12.55	-8.37	0.71042
Pot	2000	0.120	1.57	0.348	3.22	-11.36	-8.40	0.71083
Pot	2000	0.071	2.47	0.413	4.40	-12.96	-8.81	0.71075
Pot	2000	0.157	2.06	0.371	4.05	-12.29	-8.51	0.71108
Pot	2000	0.228	2.09	0.400	4.45	-11.28	-8.64	0.71079
Pot	2000	0.256	1.85	0.592	4.28	-10.82	-8.62	0.71059
Pot	2000	0.116	2.06	0.331	5.58	-14.03	-8.63	0.71089
Pot	2000	0.069	3.24	0.420	5.56	-12.23	-8.66	0.71085
Pot	2000	0.115	1.88	0.375	4.65	-13.56	-8.38	0.71096
Pot	2000	0.173	2.09	0.353	4.30	-13.80	-8.55	0.71090
Pot	2000	0.132	2.35	0.368	4.66	-14.74	-8.68	0.71097
Pot	2000	0.229	2.58	0.401	5.62	-14.25	-8.79	0.71094
Pot	2000	0.134	2.30	0.385	5.61	-13.65	-8.39	0.71087
Pot	2000	0.227	2.50	0.451	5.33	-13.07	-8.25	0.71110
Pot	2000	0.211	2.69	0.361	4.70	-14.29	-8.51	0.71084
Pot	2000	0.266	2.11	0.379	4.07	-13.51	-8.52	0.71097
Pot	2000	0.286	1.72	0.375	5.21	-14.13	-9.01	0.71098
Pot	2000	0.265	2.47	0.403	7.44	-12.17	-8.77	0.71092
Pot	2000	0.295	2.09	0.404	4.58	-12.42	-8.55	0.71090
Pot	2000	0.119	1.82	0.336	4.01	-11.42	-8.27	0.71082
Pot	2000	0.234	2.34	0.354	5.15	-14.10	-8.60	0.71091
Pot	2000	0.176	2.33	0.407	4.85	-12.66	-8.68	0.71097
Pot	2000	0.208	1.93	0.388	5.11	-12.67	-8.64	0.71107
Rap	2000	0.084	1.80	0.555	8.87	-15.33	-8.06	0.71526
Rap	2000	0.079	2.44	0.587	20.05	-15.34	-7.99	0.71561
Rap	2000	0.213	2.91	0.499	17.61	-15.78	-8.06	0.71589
Rap	2000	0.095	1.94	0.498	16.67	-15.42	-8.01	0.71561

Table A2.1 continued

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹³ C VPDB	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Rap	2000	0.160	2.54	0.480	13.84	-15.47	-8.09	0.71579
Rap	2000	0.108	2.12	0.471	14.25	-15.30	-7.77	0.71557
Rap	2000	0.093	3.20	0.482	16.83	-15.58	-8.15	0.71573
Rap	2000	0.085	3.05	0.521	15.02	-15.10	-8.03	0.71579
Rap	2000	0.075	2.69	0.553	32.91	-15.73	-8.13	0.71487
Rap	2000	0.114	2.28	0.482	9.55	-14.55	-7.94	0.71502
Rap	2000	0.120	3.11	0.502	21.43	-15.14	-7.92	0.71539
Rap	2000	0.102	3.32	0.471	21.65	-15.92	-8.22	0.71576
Rap	2000	0.083	3.00	0.508	17.86	-15.61	-8.13	0.71590
Rap	2000	0.081	2.74	0.537	13.64	-15.25	-7.94	0.71542
Rap	2000	0.079	3.17	0.552	13.01	-15.55	-8.01	0.71541
Rap	2000	0.061	3.62	0.622	15.54	-14.49	-8.00	0.72063
Rap	2000	0.128	1.50	0.428	8.36	-15.92	-9.15	0.71566
Rap	2000	0.096	2.61	0.430	9.79	-15.49	-8.98	0.71614
Rap	2000	0.089	1.16	0.473	9.46	-15.54	-9.07	0.71568
Rap	2000	0.090	2.26	0.508	6.62	-14.27	-7.95	0.71492
Rap	2000	0.072	2.12	0.521	20.80	-15.09	-8.02	0.71558
Mat	2000	0.036	4.10	0.709	11.80	-17.05	-6.79	0.71152
Mat	2000	0.031	7.30	0.826	18.14	-17.58	-6.89	0.71208
Mat	2000	0.035	5.58	0.835	18.27	-16.42	-6.78	0.71134
Mat	2000	0.037	5.78	0.855	18.05	-17.25	-7.09	0.71166
Mat	2000	0.028	5.15	0.699	15.70	-17.96	-7.09	0.71236
Mat	2000	0.042	7.16	0.799	24.02	-18.58	-7.04	0.71292
Mat	2000	0.047	6.10	0.705	14.42	-16.74	-6.91	0.71204
Mat	2000	0.012	7.23	0.683	22.37	-17.15	-6.91	0.71222
Mat	2000	0.040	5.38	0.829	13.09	-16.67	-6.81	0.71129
Mat	2000	0.031	5.16	0.580	11.90	-17.75	-7.02	0.71231
Mat	2000	0.060	6.00	0.705	13.00	-18.63	-7.21	0.71240
Mat	2000	0.030	5.36	0.722	18.49	-18.47	-7.01	0.71232
Mat	2000	0.040	4.98	0.775	18.04	-17.58	-7.04	0.71170
Mat	2000	0.040	4.67	0.804	13.37	-16.63	-7.22	0.71119

Table A2.1 continued

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{13}\text{C}$ VPDB	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
Mat	2000	0.049	5.47	0.876	18.36	-17.45	-7.04	0.71202
Mat	2000	0.037	6.02	0.766	22.56	-17.89	-6.99	0.71255
Mat	2000	0.036	5.77	0.771	24.23	-17.80	-6.97	0.71233
Mat	2000	0.031	5.76	0.818	17.64	-18.51	-7.18	0.71274
Mat	2000	0.047	5.49	0.776	19.09	-17.73	-7.09	0.71214
Mat	2000	0.039	6.68	0.805	19.05	-17.56	-7.17	0.71224
Mat	2000	0.049	5.16	0.681	17.53	-17.26	-6.92	0.71251
Mat	2000	0.047	6.06	0.693	17.52	-16.49	-7.07	0.71147
Mat	2000	0.058	5.90	0.760	23.09	-16.97	-6.88	0.71265
Mat	2000	0.066	5.55	0.699	19.62	-17.19	-7.01	0.71239
Mat	2000	0.055	4.68	0.766	15.30	-19.44	-7.48	0.71197
Mat	2000	0.042	6.53	0.760	24.86	-16.74	-6.89	0.71221
Mat	2000	0.038	4.93	0.715	25.74	-17.38	-6.73	0.71267
Mat	2001	0.097	3.73	0.526	12.70	-18.10	-6.89	0.71245
Mat	2001	0.041	2.73	0.577	16.80	-18.77	-6.66	0.71268
Mat	2001	0.088	4.27	0.592	12.67	-17.97	-6.68	0.71222
Mat	2001	0.065	3.80	0.536	13.55	-18.30	-6.61	0.71254
Mat	2001	0.054	2.92	0.639	13.65	-18.13	-6.66	0.71240
Mat	2001	0.108	4.12	0.578	13.26	-17.97	-6.68	0.71229
Mat	2001	0.070	6.51	0.642	15.61	-18.94	-6.89	0.71265
Mat	2001	0.077	3.38	0.530	12.97	-16.75	-6.49	0.71209
Mat	2001	0.081	3.79	0.607	13.08	-18.43	-6.62	0.71225
Mat	2001	0.070	2.51	0.551	9.40	-17.27	-6.37	0.71181
Mat	2001	0.090	3.14	0.500	11.98	-17.69	-6.45	0.71200
Mat	2001	0.140	4.33	0.563	14.76	-17.36	-6.63	0.71227
Mat	2001	0.088	3.83	0.548	12.35	-16.86	-6.76	0.71199
Mat	2001	0.063	3.30	0.633	11.09	-16.52	-6.61	0.71164
Mat	2001	0.172	3.23	0.568	11.35	-17.89	-6.60	0.71196
Mat	2001	0.085	2.42	0.651	25.10	-17.74	-6.60	0.71282
Mat	2001	0.062	6.01	0.624	17.23	-18.05	-6.57	0.71276
Mat	2001	0.058	2.49	0.572	11.99	-17.86	-6.62	0.71220

Table A2.1 continued

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{13}\text{C}$ VPDB	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
Mat	2001	0.118	3.91	0.562	12.97	-18.41	-6.54	0.71228
Mat	2001	0.084	3.04	0.618	18.53	-18.55	-6.67	0.71257
Mat	2001	0.093	4.55	0.597	12.78	-18.27	-6.72	0.71223
Mat	2001	0.095	2.53	0.576	11.54	-17.89	-6.64	0.71178
Mat	2001	0.077	2.97	0.520	14.38	-18.31	-6.76	0.71239
Mat	2001	0.145	3.51	0.536	14.65	-19.00	-6.86	0.71213
Mat	2001	0.141	3.92	0.571	14.42	-17.57	-6.51	0.71222
Mat	2001	0.086	4.11	0.487	12.50	-17.38	-6.59	0.71205
Mat	2001	0.104	4.50	0.564	13.53	-17.59	-6.69	0.71242
Mat	2001	0.124	3.03	0.557	14.60	-18.84	-6.87	0.71218
Mat	2002	0.081	3.87	0.758	15.10	-18.99	-5.51	0.71234
Mat	2002	0.103	4.19	0.709	15.08	-19.06	-5.55	0.71240
Mat	2002	0.069	5.98	0.742	18.03	-18.99	-5.74	0.71234
Mat	2002	0.085	2.86	0.588	14.40	-18.14	-5.35	0.71221
Mat	2002	0.091	2.54	0.671	16.60	-18.73	-5.36	0.71172
Mat	2002	0.082	4.53	0.699	17.93	-18.50	-5.44	0.71240
Mat	2002	0.099	2.68	0.579	15.38	-18.72	-5.48	0.71201
Mat	2002	0.128	3.88	0.593	11.45	-18.41	-5.52	0.71183
Mat	2002	0.095	3.26	0.603	12.57	-17.84	-5.32	0.71217
Mat	2002	0.074	2.99	0.664	16.82	-17.09	-5.65	0.71131
Mat	2002	0.073	6.94	0.863	17.69	-18.79	-5.94	0.71261
Mat	2002	0.091	7.64	0.750	16.78	-18.90	-5.68	0.71249
Mat	2002	0.119	6.49	0.714	20.10	-18.80	-5.62	0.71249
Mat	2002	0.129	4.61	0.641	17.60	-18.50	-6.03	0.71267
Mat	2002	0.091	8.23	0.861	16.45	-18.63	-5.61	0.71263
Mat	2002	0.098	7.03	0.721	14.46	-18.80	-5.69	0.71245
Mat	2002	0.074	7.43	0.877	16.15	-18.71	-5.73	0.71255
Mat	2002	0.089	5.35	0.734	16.72	-18.81	-5.69	0.71251
Mat	2002	0.079	4.28	0.677	14.56	-19.03	-5.62	0.71250
Mat	2002	0.122	4.19	0.581	17.34	-18.38	-5.40	0.71230
Mat	2002	0.091	3.94	0.693	12.41	-18.72	-5.40	0.71176

Table A2.1 continued

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{13}\text{C}$ VPDB	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
Mat	2002	0.085	3.67	0.712	18.77	-18.61	-5.39	0.71215
Mat	2002	0.100	3.18	0.754	16.55	-18.61	-5.53	0.71208
Mat	2002	0.072	3.41	0.709	15.25	-18.55	-5.60	0.71185
Pam	2000	0.049	4.02	0.603	26.75	-16.45	-6.51	0.71231
Pam	2000	0.056	3.96	0.537	19.43	-16.68	-6.47	0.71231
Pam	2000	0.048	2.98	0.511	11.80	-16.55	-6.34	0.71229
Pam	2000	0.010	3.70	0.670	26.51	-16.72	-6.49	0.71232
Pam	2000	0.011	4.21	0.525	14.79	-16.80	-6.57	0.71247
Pam	2000	0.069	5.03	0.505	18.82	-16.53	-6.37	0.71232
Pam	2000	0.018	5.10	0.685	12.68	-16.61	-6.68	0.71233
Pam	2000	0.044	6.01	0.698	13.99	-17.78	-7.07	0.71212
Pam	2000	0.104	3.73	0.537	19.33	-16.64	-6.65	0.71222
Pam	2000	0.051	3.26	0.544	19.43	-16.88	-6.56	0.71224
Pam	2000	0.053	6.41	0.698	16.04	-16.53	-6.89	0.71157
Pam	2000	0.043	3.50	0.503	17.47	-17.04	-6.67	0.71265
Pam	2000	0.032	4.30	0.596	14.05	-17.01	-6.72	0.71219
Pam	2000	0.034	3.90	0.574	15.53	-16.88	-6.63	0.71237
Pam	2000	0.150	4.60	0.473	12.31	-16.28	-6.70	0.71227
Pam	2000	0.067	2.60	0.495	17.83	-16.55	-6.42	0.71229
Pam	2000	0.035	3.06	0.593	19.37	-16.24	-6.34	0.71227
Pam	2000	0.032	4.29	0.529	16.13	-16.81	-6.70	0.71242
Pam	2001	0.086	3.66	0.545	14.64	-17.03	-6.30	0.71215
Pam	2001	0.237	2.18	0.446	12.52	-16.92	-6.20	0.71211
Pam	2001	0.128	2.17	0.547	16.01	-16.88	-6.45	0.71224
Pam	2001	0.210	3.01	0.463	11.59	-15.99	-6.27	0.71218
Pam	2001	0.162	3.42	0.574	16.19	-16.56	-6.28	0.71217
Pam	2001	0.247	3.65	0.489	13.27	-17.18	-6.53	0.71193
Pam	2001	0.293	2.46	0.401	9.22	-16.90	-6.46	0.71188
Pam	2001	0.115	2.33	0.475	13.48	-17.05	-6.36	0.71221
Pam	2001	0.187	1.65	0.405	12.33	-16.24	-6.31	0.71217
Pam	2001	0.268	2.25	0.394	9.95	-15.77	-6.30	0.71215

Table A2.1 continued

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹³ C VPDB	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Pam	2001	0.114	3.82	0.546	12.38	-17.51	-6.54	0.71194
Pam	2001	0.209	3.53	0.503	14.34	-15.92	-6.14	0.71220
Pam	2001	0.207	2.48	0.474	12.96	-16.14	-6.36	0.71223
Pam	2001	0.186	2.04	0.413	11.08	-16.85	-6.46	0.71219
Pam	2001	0.226	2.05	0.449	11.95	-17.08	-6.53	0.71201
Pam	2001	0.240	2.85	0.449	11.14	-16.58	-6.34	0.71207
Pam	2001	0.166	2.76	0.522	13.33	-16.74	-6.37	0.71209
Pam	2001	0.096	2.60	0.518	12.94	-17.00	-6.32	0.71194
Pam	2001	0.185	3.49	0.471	11.84	-16.66	-6.52	0.71201
Pam	2001	0.140	2.26	0.457	10.92	-15.89	-6.05	0.71227
Pam	2001	0.095	2.50	0.500	16.69	-16.85	-6.31	0.71211
Pam	2001	0.212	4.05	0.618	18.35	-16.74	-6.63	0.71225
Pam	2001	0.089	2.22	0.495	12.34	-16.67	-6.13	0.71218
Pam	2001	0.193	3.16	0.533	9.37	-16.57	-6.32	0.71169
Pam	2001	0.078	2.37	0.494	12.40	-16.39	-6.02	0.71221
Pam	2001	0.143	2.75	0.526	11.11	-17.53	-6.60	0.71215
Pam	2001	0.130	3.44	0.472	13.86	-16.90	-6.11	0.71255
Pam	2001	0.173	2.90	0.480	13.05	-16.25	-6.13	0.71225
Pam	2001	0.139	3.59	0.603	12.27	-16.98	-6.31	0.71215
Pam	2002	0.099	5.81	0.459	7.86	-18.36	-5.42	0.71211
Pam	2002	0.088	3.37	0.428	11.06	-16.77	-5.22	0.71222
Pam	2002	0.151	6.16	0.425	9.11	-17.57	-5.91	0.71199
Pam	2002	0.087	3.07	0.386	7.17	-16.57	-5.17	0.71211
Pam	2002	0.104	4.04	0.525	7.70	-17.08	-5.14	0.71211
Pam	2002	0.083	3.87	0.494	8.06	-16.47	-5.10	0.71212
Pam	2002	0.084	4.30	0.447	7.67	-17.34	-4.93	0.71208
Pam	2002	0.081	4.62	0.369	7.50	-17.35	-4.66	0.71196
Pam	2002	0.144	4.37	0.431	10.63	-17.03	-5.08	0.71216
Pam	2002	0.096	4.56	0.370	9.38	-17.71	-4.91	0.71194
Pam	2002	0.106	6.46	0.474	9.90	-17.34	-4.82	0.71197
Pam	2002	0.110	5.62	0.456	8.95	-17.32	-5.00	0.71198

Table A2.1 continued

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{13}\text{C}$ VPDB	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
Pam	2002	0.115	5.49	0.494	10.02	-18.35	-5.26	0.71186
Pam	2002	0.106	4.41	0.468	9.31	-18.05	-4.90	0.71191
Pam	2002	0.092	6.30	0.419	7.46	-17.82	-4.98	0.71194
Pam	2002	0.099	4.73	0.527	7.39	-18.60	-5.27	0.71196
Pam	2002	0.088	4.43	0.502	6.26	-17.90	-5.36	0.71170
Pam	2002	0.101	4.31	0.505	8.48	-18.30	-5.10	0.71184
Pam	2002	0.088	5.58	0.729	9.41	-17.98	-5.21	0.71177
StC	2000	0.146	4.81	1.168	18.31	-16.73	-5.07	0.70956
StC	2000	0.082	2.40	1.428	16.73	-16.94	-5.64	0.70956
StC	2000	0.078	2.33	1.168	16.73	-15.90	-4.90	0.70954
StC	2000	0.085	3.05	1.277	21.96	-16.89	-5.54	0.70954
StC	2000	0.077	3.17	1.238	26.01	-15.50	-5.12	0.70962
StC	2000	0.092	1.88	1.073	19.24	-16.11	-5.81	0.70953
StC	2000	0.318	2.27	1.740	32.55	-14.68	-5.41	0.70955
StC	2000	0.095	3.15	1.699	24.87	-16.29	-5.42	0.70951
StC	2000	0.211	1.75	1.185	23.07	-14.78	-5.86	0.70943
StC	2000	0.111	1.96	1.331	25.44	-16.07	-5.72	0.70948
StC	2000	0.209	2.32	1.531	19.22	-17.32	-5.43	0.70954
StC	2000	0.088	2.52	2.472	55.66	-14.17	-5.70	0.70949
StC	2000	0.087	4.31	1.519	22.13	-15.64	-4.97	0.70953
StC	2000	0.160	3.07	0.706	15.17	-17.42	-5.87	0.70951
StC	2000	0.088	1.75	1.202	31.97	-15.17	-6.03	0.71020
StC	2000	0.133	2.23	2.863	52.46	-15.01	-5.35	0.70947
StC	2000	0.169	1.73	1.943	33.01	-16.72	-5.24	0.70946
StC	2000	0.108	3.04	0.998	20.27	-17.06	-4.92	0.70953
StC	2000	0.151	1.51	1.167	21.38	-17.19	-5.28	0.70946
StC	2000	0.081	3.80	0.954	13.07	-17.65	-6.19	0.70960
StC	2000	0.103	2.58	1.253	15.64	-16.90	-5.64	0.70955
StC	2000	0.133	2.11	2.426	38.71	-14.71	-5.78	0.70949
StC	2000	0.140	1.98	1.825	41.96	-15.80	-5.87	0.70943
StC	2000	0.094	3.17	2.009	37.44	-15.38	-6.03	0.70946

Table A2.1 continued

River	Year	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹³ C VPDB	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
StC	2000	0.096	3.47	1.715	24.37	-17.24	-5.25	0.70957
StC	2000	0.106	3.84	1.294	20.02	-16.27	-5.58	0.70954
Alt	2000	0.082	2.70	0.413	13.89	-17.14	-5.11	0.71112
Alt	2000	0.166	2.90	0.291	11.46	-15.10	-5.01	0.71132
Alt	2000	0.070	3.48	1.179	32.33	-13.91	-4.97	0.71042
Alt	2000	0.147	3.80	0.532	14.31	-14.57	-5.04	0.71258
Alt	2000	0.051	2.65	0.418	20.22	-16.95	-4.95	0.71097
Alt	2000	0.103	1.63	0.425	12.55	-17.22	-5.20	0.71098
Alt	2000	0.060	2.05	0.408	11.16	-16.77	-4.97	0.71084
Alt	2000	0.079	3.85	0.509	17.39	-16.01	-5.03	0.71080
Alt	2000	0.129	1.65	0.288	12.61	-17.40	-5.03	0.71084
Alt	2000	0.063	2.79	0.436	15.07	-16.95	-4.87	0.71116
Alt	2000	0.147	3.87	0.380	15.35	-16.94	-4.86	0.71121
Alt	2000	0.138	2.59	0.354	9.44	-16.64	-4.91	0.71118
Alt	2000	0.055	3.38	0.476	11.21	-17.30	-5.03	0.71099
Alt	2000	0.078	2.39	0.261	8.34	-16.17	-5.86	0.71164
Alt	2000	0.088	2.51	0.344	12.33	-17.31	-5.31	0.71100
Alt	2000	0.114	5.02	0.407	20.38	-17.15	-5.14	0.71073
Alt	2000	0.214	1.96	0.396	12.30	-16.64	-4.82	0.71111
Alt	2000	0.072	5.40	0.420	12.42	-16.38	-4.98	0.71123
Alt	2000	0.163	1.96	0.446	11.58	-17.37	-5.27	0.71086
Alt	2000	0.303	2.01	0.283	9.34	-16.72	-5.24	0.71110
Alt	2000	0.079	2.89	0.370	9.95	-17.06	-4.92	0.71089
Alt	2000	0.072	3.23	0.477	31.08	-17.19	-5.28	0.71104
Alt	2000	0.111	1.44	0.232	6.39	-15.55	-4.97	0.71124
Alt	2000	0.094	2.00	0.313	12.58	-17.34	-5.04	0.71793

APPENDIX 3

ANALYTICAL DATA FOR SAMPLES USED IN CHAPTER 3

Table A3.1 Juvenile American shad collected from rivers in 2004 and used for analyses reported in Chapter 3. Each row reports corrected elemental and isotopic ratios obtained from the otoliths of one individual fish. River codes: Ken: Kennebec River, ME. Exe: Exeter River, NH. Con: Connecticut River, CT. Hud: Hudson River, NY. Del: Delaware River, NJ. UpC: Upper Chesapeake Bay, MD. Pot: Potomac River, MD. Rap: Rappahannock River, VA. Mat: Mattaponi River, VA. Roa: Roanoke River, NC. StC: Santee-Cooper River, SC. Alt: Altamaha River, GA. SJs: St. Johns River, Florida

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Ken	0.064	15.09	0.416	4.38	-10.28	0.71368
Ken	0.107	17.89	0.419	5.27	-10.01	0.71380
Ken	0.094	19.66	0.458	3.58	-10.16	0.71374
Ken	0.074	18.08	0.453	3.42	-9.82	0.71358
Ken	0.073	31.59	0.458	4.35	-10.35	0.71361
Ken	0.071	14.68	0.386	3.44	-10.07	0.71376
Ken	0.071	21.08	0.537	4.08	-10.13	0.71379
Ken	0.098	12.90	0.448	6.26	-10.18	0.71383
Ken	0.090	18.04	0.447	4.76	-10.37	0.71373
Ken	0.084	14.96	0.487	4.27	-9.86	0.71377
Ken	0.138	24.42	0.385	5.13	-10.91	0.71381
Ken	0.103	17.06	0.393	4.57	-11.10	0.71384
Ken	0.073	13.58	0.416	4.20	-10.82	0.71379
Ken	0.114	19.28	0.529	6.03	-11.04	0.71378
Ken	0.142	18.44	0.427	4.34	-11.07	0.71388
Ken	0.103	10.56	0.420	4.22	-10.58	0.71364
Ken	0.082	17.35	0.434	3.69	-10.35	0.71378
Ken	0.068	13.53	0.495	4.61	-10.12	0.71348
Ken	0.056	13.08	0.449	5.50	-10.22	0.71383
Ken	0.067	13.65	0.473	5.68	-9.99	0.71379
Ken	0.071	15.44	0.457	4.29	-10.59	0.71380
Ken	0.048	15.34	0.490	5.27	-10.43	0.71379
Ken	0.091	14.35	0.441	3.60	-10.16	0.71346

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
Ken	0.069	13.79	0.467	4.74	-10.41	0.71365
Ken	0.059	12.85	0.505	6.63	-10.01	0.71383
Ken	0.084	21.25	0.437	4.31	-10.18	0.71378
Ken	0.078	12.46	0.423	4.67	-10.23	0.71374
Ken	0.094	12.58	0.404	5.10	-9.84	0.71379
Ken	0.086	13.86	0.433	4.19	-11.25	0.71375
Ken	0.161	13.98	0.441	5.06	-10.64	0.71373
Ken	0.212	21.52	0.449	4.85	-10.21	0.71370
Ken	0.077	15.18	0.401	5.16	-10.81	0.71380
Ken	0.110	13.93	0.419	4.59	-10.83	0.71368
Ken	0.146	18.83	0.417	5.00	-10.52	0.71373
Ken	0.042	11.81	0.479	5.59	-10.57	0.71372
Ken	0.130	30.54	0.435	4.52	-10.85	0.71377
Ken	0.061	16.96	0.458	4.12	-10.87	0.71375
Ken	0.072	11.17	0.339	4.35	-10.48	0.71367
Ken	0.185	15.73	0.432	4.70	-10.49	0.71373
Ken	0.069	9.75	0.444	4.60	-10.52	0.71368
Ken	0.086	19.67	0.487	4.48	-10.68	0.71357
Ken	0.076	13.23	0.488	6.12	-10.17	0.71371
Ken	0.074	16.80	0.459	3.52	-10.14	0.71355
Ken	0.204	20.58	0.504	5.46	-10.39	0.71353
Ken	0.116	9.92	0.481	4.00	-10.09	0.71359
Ken	0.053	14.26	0.497	4.10	-10.02	0.71356
Ken	0.104	14.50	0.500	6.04	-10.22	0.71366
Ken	0.077	11.84	0.475	4.21	-9.85	0.71372
Ken	0.083	10.45	0.487	3.55	-9.89	0.71355
Ken	0.144	10.60	0.459	3.91	-10.20	0.71373
Ken	0.091	19.35	0.431	4.16	-11.04	0.71371
Ken	0.091	9.73	0.340	3.03	-10.95	0.71366
Ken	0.140	15.57	0.431	4.01	-10.52	0.71361
Ken	0.157	16.44	0.467	3.73	-10.94	0.71368
Ken	0.093	28.88	0.464	4.62	-11.01	0.71371

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
Ken	0.126	11.63	0.373	4.11	-10.66	0.71391
Ken	0.105	13.66	0.335	4.11	-11.01	0.71376
Ken	0.099	18.40	0.437	4.16	-11.01	0.71373
Exe	0.066	19.59	0.407	5.82	-7.80	0.71705
Exe	0.150	18.93	0.482	8.43	-8.20	0.71698
Exe	0.095	24.45	0.504	6.27	-7.62	0.71715
Exe	0.130	22.55	0.483	8.32	-7.20	0.71698
Exe	0.038	25.51	0.623	11.46	-7.58	0.71721
Exe	0.085	27.39	0.512	9.41	-7.75	0.71711
Exe	0.139	13.56	0.509	7.08	-7.90	0.71693
Exe	0.062	22.13	0.501	7.03	-7.75	0.71708
Exe	0.107	19.14	0.532	9.38	-7.53	0.71715
Exe	0.130	15.33	0.500	8.43	-7.64	0.71705
Exe	0.094	18.88	0.495	7.99	-7.69	0.71691
Exe	0.126	18.94	0.457	7.82	-7.63	0.71691
Exe	0.069	13.04	0.525	8.90	-7.60	0.71716
Exe	0.059	21.78	0.472	7.43	-7.74	0.71723
Exe	0.050	16.09	0.549	9.65	-7.72	0.71685
Exe	0.090	16.47	0.578	9.02	-7.67	0.71714
Exe	0.138	17.45	0.522	7.02	-7.38	0.71697
Exe	0.141	21.13	0.515	8.77	-7.58	0.71694
Exe	0.063	23.94	0.552	10.37	-7.66	0.71715
Exe	0.085	24.21	0.506	9.94	-7.69	0.71704
Exe	0.096	24.29	0.514	7.36	-7.72	0.71706
Exe	0.103	17.12	0.547	10.00	-7.83	0.71692
Exe	0.109	29.36	0.430	4.44	-7.92	0.71702
Exe	0.101	12.42	0.500	7.95	-7.98	0.71688
Exe	0.143	12.00	0.497	8.58	-8.11	0.71706
Exe	0.183	18.88	0.593	12.15	-7.83	0.71705
Exe	0.066	17.73	0.514	6.39	-8.03	0.71672
Exe	0.123	23.14	0.434	5.15	-7.89	0.71656
Exe	0.128	19.36	0.487	7.28	-7.92	0.71711

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ¹⁸O VPDB	⁸⁷Sr/⁸⁶Sr
Exe	0.201	12.35	0.548	8.07	-7.75	0.71702
Exe	0.129	26.61	0.547	7.84	-7.69	0.71712
Exe	0.114	24.11	0.524	7.96	-7.76	0.71712
Exe	0.149	16.73	0.402	5.17	-7.90	0.71702
Exe	0.100	21.88	0.533	8.88	-7.70	0.71692
Exe	0.088	21.70	0.509	8.33	-7.75	0.71695
Exe	0.151	15.34	0.518	4.98	-7.89	0.71713
Exe	0.210	20.73	0.486	8.84	-8.14	0.71689
Exe	0.246	13.22	0.442	5.07	-7.96	0.71698
Exe	0.130	19.91	0.475	7.82	-8.05	0.71694
Exe	0.267	12.77	0.449	7.29	-7.64	0.71697
Exe	0.127	25.59	0.439	6.07	-7.83	0.71704
Con	0.092	7.58	0.437	4.74	-11.33	0.71280
Con	0.150	14.27	0.434	5.37	-11.57	0.71283
Con	0.210	14.93	0.430	5.47	-11.40	0.71282
Con	0.103	12.43	0.441	7.20	-11.40	0.71285
Con	0.068	12.93	0.429	7.86	-11.17	0.71285
Con	0.086	10.01	0.446	4.78	-10.96	0.71318
Con	0.056	10.66	0.377	4.64	-11.38	0.71293
Con	0.099	12.84	0.406	5.36	-11.15	0.71285
Con	0.076	19.61	0.466	7.09	-11.31	0.71267
Con	0.063	19.25	0.408	7.18	-11.38	0.71272
Con	0.079	17.93	0.409	4.35	-11.50	0.71236
Con	0.075	15.74	0.431	7.10	-11.55	0.71282
Con	0.117	16.51	0.408	5.46	-11.27	0.71288
Con	0.111	18.11	0.402	7.97	-10.90	0.71280
Con	0.063	17.16	0.429	5.58	-11.51	0.71276
Con	0.058	10.63	0.426	4.64	-11.44	0.71290
Con	0.063	11.83	0.532	14.64	-10.84	0.71314
Con	0.094	13.40	0.677	19.68	-10.66	0.71297
Con	0.089	13.89	0.608	22.80	-10.94	0.71270
Con	0.067	14.18	0.777	17.32	-10.57	0.71311

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Con	0.123	8.28	0.498	6.98	-10.78	0.71314
Con	0.095	14.20	0.486	7.91	-10.74	0.71279
Con	0.070	14.53	0.580	13.49	-10.74	0.71323
Con	0.141	11.85	0.532	8.90	-10.87	0.71313
Con	0.088	21.89	0.415	5.67	-10.99	0.71298
Con	0.138	13.17	0.529	12.23	-10.77	0.71293
Con	0.097	9.06	0.505	10.55	-9.73	0.71292
Con	0.127	11.04	0.491	7.72	-10.50	0.71330
Con	0.073	14.05	0.504	10.65	-10.99	0.71319
Con	0.090	15.32	0.514	8.60	-10.79	0.71317
Con	0.078	10.86	0.517	10.55	-10.12	0.71393
Con	0.102	8.81	0.505	10.63	-11.06	0.71321
Con	0.130	11.73	0.503	7.13	-10.90	0.71311
Con	0.099	10.55	0.492	7.48	-10.90	0.71303
Con	0.080	9.26	0.486	7.39	-10.52	0.71345
Con	0.108	22.76	0.417	5.23	-11.38	0.71266
Con	0.078	30.29	0.422	7.52	-11.52	0.71274
Con	0.073	16.72	0.477	10.43	-10.18	0.71274
Con	0.076	14.82	0.469	5.74	-11.50	0.71262
Con	0.075	14.07	0.480	7.10	-11.16	0.71293
Con	0.071	13.28	0.404	6.11	-11.31	0.71301
Con	0.070	16.86	0.419	6.96	-11.20	0.71287
Con	0.070	10.76	0.378	4.56	-11.31	0.71288
Con	0.119	22.02	0.572	10.50	-11.08	0.71289
Con	0.091	14.87	0.508	8.96	-10.43	0.71292
Con	0.064	17.36	0.475	7.24	-10.95	0.71283
Con	0.103	10.75	0.509	11.75	-10.67	0.71341
Con	0.174	6.34	0.511	8.65	-10.58	0.71352
Con	0.077	6.42	0.464	10.48	-10.69	0.71335
Con	0.209	11.67	0.653	15.42	-10.64	0.71298
Con	0.085	10.44	0.571	8.00	-10.80	0.71317
Hud	0.094	8.01	0.358	5.20	-10.53	0.71131

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ¹⁸O VPDB	⁸⁷Sr/⁸⁶Sr
Hud	0.139	16.92	0.381	5.03	-10.48	0.71132
Hud	0.086	7.99	0.530	7.42	-10.95	0.71073
Hud	0.047	7.87	0.428	5.78	-9.87	0.71075
Hud	0.060	9.52	0.439	6.36	-8.55	0.71097
Hud	0.096	7.46	0.376	5.00	-10.71	0.71158
Hud	0.066	6.63	0.484	6.98	-10.44	0.71064
Hud	0.055	6.60	0.412	4.33	-9.66	0.71153
Hud	0.078	9.52	0.446	4.31	-10.43	0.71035
Hud	0.067	9.75	0.428	5.85	-10.69	0.71126
Hud	0.047	3.54	0.461	5.87	-10.54	0.71076
Hud	0.039	3.96	0.447	5.38	-10.37	0.71157
Hud	0.052	9.86	0.398	6.06	-9.41	0.71111
Hud	0.078	5.30	0.371	4.63	-8.68	0.71156
Hud	0.082	9.74	0.411	6.03	-10.34	0.71150
Hud	0.076	9.94	0.436	6.25	-11.22	0.71103
Hud	0.060	8.46	0.409	5.71	-11.00	0.71141
Hud	0.077	5.42	0.448	6.93	-9.58	0.71146
Hud	0.117	5.36	0.446	4.82	-10.71	0.71077
Hud	0.055	10.14	0.487	6.45	-10.94	0.71024
Hud	0.054	5.76	0.510	6.90	-10.25	0.71078
Hud	0.048	9.41	0.492	6.24	-10.86	0.71049
Hud	0.059	5.20	0.499	4.61	-10.77	0.71036
Hud	0.076	3.45	0.497	4.09	-10.87	0.71047
Hud	0.054	8.84	0.503	5.43	-10.80	0.71060
Hud	0.058	11.07	0.441	6.55	-11.37	0.71092
Hud	0.075	11.73	0.439	9.75	-11.16	0.71029
Hud	0.062	9.71	0.483	5.02	-10.62	0.71041
Hud	0.103	10.07	0.411	6.58	-11.10	0.71075
Hud	0.071	9.43	0.410	5.89	-10.98	0.71088
Hud	0.039	2.91	0.617	6.22	-11.02	0.71077
Hud	0.049	2.67	0.646	7.56	-11.13	0.71051
Hud	0.104	10.13	0.433	5.25	-10.91	0.71060

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Hud	0.090	9.70	0.456	4.86	-11.26	0.71093
Hud	0.111	7.02	0.497	5.93	-10.64	0.71088
Hud	0.190	7.34	0.721	3.18	-10.95	0.70942
Hud	0.092	9.90	0.451	6.64	-9.01	0.71059
Hud	0.084	7.18	0.367	3.89	-10.93	0.71090
Hud	0.143	7.67	0.378	3.50	-10.32	0.71120
Hud	0.115	9.96	0.391	6.58	-10.29	0.71099
Hud	0.063	9.29	0.465	5.60	-10.52	0.71126
Hud	0.085	10.81	0.405	5.11	-10.51	0.71125
Hud	0.096	7.03	0.361	7.03	-10.67	0.71136
Hud	0.080	11.30	0.380	6.07	-11.07	0.71127
Hud	0.082	10.71	0.400	5.55	-10.34	0.71151
Hud	0.079	10.52	0.467	9.05	-10.87	0.71129
Hud	0.097	9.39	0.453	8.52	-11.12	0.71081
Hud	0.071	9.43	0.391	6.58	-10.56	0.71122
Hud	0.060	11.28	0.419	5.22	-10.55	0.71135
Hud	0.073	13.80	0.417	7.90	-10.56	0.71165
Hud	0.065	9.43	0.410	6.25	-11.05	0.71044
Hud	0.084	9.74	0.389	5.28	-11.07	0.71080
Hud	0.069	6.43	0.413	4.40	-10.40	0.71127
Hud	0.074	7.76	0.377	5.90	-10.86	0.71033
Del	0.084	22.55	0.425	12.33	-10.13	0.71218
Del	0.059	12.21	0.392	14.46	-10.41	0.71289
Del	0.054	23.01	0.414	19.02	-10.32	0.71340
Del	0.069	31.49	0.392	21.38	-10.11	0.71343
Del	0.065	29.37	0.561	23.66	-10.37	0.71211
Del	0.078	28.58	0.529	27.41	-10.17	0.71249
Del	0.057	43.85	0.579	21.59	-10.08	0.71194
Del	0.077	19.83	0.534	21.70	-10.12	0.71211
Del	0.067	20.03	0.382	29.58	-10.00	0.71404
Del	0.114	30.60	0.536	19.03	-9.99	0.71199
Del	0.075	23.13	0.440	14.14	-9.78	0.71214

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Del	0.199	29.21	0.500	20.28	-9.85	0.71203
Del	0.076	22.63	0.407	17.07	-10.35	0.71318
Del	0.065	38.64	0.504	18.85	-9.96	0.71227
Del	0.157	14.77	0.365	9.23	-10.25	0.71204
Del	0.090	13.72	0.427	13.11	-10.37	0.71215
Del	0.136	33.06	0.408	23.35	-10.22	0.71337
Del	0.117	18.25	0.536	19.37	-10.24	0.71214
Del	0.098	32.29	0.515	25.15	-9.93	0.71330
Del	0.094	41.98	0.411	22.40	-9.85	0.71356
Del	0.081	29.65	0.418	34.44	-10.11	0.71407
Del	0.079	29.01	0.379	33.23	-10.24	0.71394
Del	0.090	28.41	0.496	24.85	-10.00	0.71367
Del	0.081	16.96	0.349	21.12	-10.66	0.71392
Del	0.094	78.83	0.508	44.62	-9.86	0.71311
Del	0.060	12.18	0.374	25.04	-10.06	0.71388
Del	0.093	44.96	0.507	26.16	-10.30	0.71332
Del	0.086	26.58	0.443	25.54	-10.17	0.71360
Del	0.087	25.89	0.475	24.66	-10.58	0.71348
Del	0.149	32.95	0.369	27.94	-10.01	0.71416
Del	0.105	12.60	0.413	33.41	-10.04	0.71415
Del	0.128	26.56	0.428	20.61	-10.35	0.71336
Del	0.062	32.18	0.479	29.51	-10.11	0.71370
Del	0.111	10.59	0.410	20.20	-10.62	0.71407
Del	0.113	43.49	0.407	29.88	-9.59	0.71404
Del	0.076	20.98	0.335	17.50	-10.07	0.71426
Del	0.127	23.98	0.340	16.29	-10.29	0.71349
Del	0.064	17.56	0.433	25.72	-10.39	0.71407
Del	0.071	31.71	0.480	25.44	-10.07	0.71310
Del	0.093	29.40	0.497	18.25	-10.44	0.71216
Del	0.082	17.83	0.422	21.57	-10.28	0.71357
Del	0.127	30.52	0.511	25.62	-10.33	0.71267
Del	0.078	22.55	0.399	32.84	-10.34	0.71395

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Del	0.180	9.52	0.434	12.28	-10.43	0.71242
Del	0.085	27.61	0.440	23.76	-10.16	0.71324
Del	0.094	23.80	0.474	25.50	-9.81	0.71348
Del	0.100	20.21	0.468	17.90	-10.41	0.71210
Del	0.141	17.42	0.361	18.49	-10.21	0.71336
Del	0.077	27.14	0.466	22.18	-10.01	0.71336
Del	0.075	32.97	0.516	23.17	-10.16	0.71213
Del	0.077	29.95	0.471	27.72	-10.27	0.71349
Del	0.086	11.61	0.663	7.01	-9.79	0.71182
Del	0.041	7.38	0.400	17.11	-10.38	0.71347
Del	0.094	9.22	0.449	7.77	-9.38	0.71252
Del	0.062	14.55	0.413	7.41	-9.79	0.71211
Del	0.109	56.64	0.519	25.51	-9.98	0.71195
Del	0.106	33.04	0.485	32.81	-10.13	0.71348
UpC	0.084	10.11	0.663	4.46	-9.77	0.71080
UpC	0.117	9.67	0.794	5.18	-9.80	0.71138
UpC	0.170	9.77	0.680	6.11	-9.89	0.71060
UpC	0.070	7.89	0.899	4.94	-9.63	0.71087
UpC	0.123	5.24	0.925	5.02	-9.54	0.71095
UpC	0.071	12.67	0.593	5.53	-10.09	0.71094
UpC	0.099	4.67	0.566	3.87	-10.10	0.71050
UpC	0.094	9.28	0.716	5.44	-10.03	0.71123
UpC	0.104	5.15	0.438	7.13	-9.54	0.71174
UpC	0.153	12.39	0.400	6.88	-10.42	0.71188
UpC	0.115	10.70	0.831	4.99	-9.99	0.71056
UpC	0.104	10.26	0.779	5.74	-9.41	0.71066
UpC	0.106	12.90	0.839	4.14	-9.51	0.71097
UpC	0.064	11.97	0.664	5.12	-10.31	0.71112
UpC	0.098	5.67	0.619	3.61	-10.03	0.71038
UpC	0.060	15.56	0.395	13.06	-9.96	0.71191
UpC	0.104	13.40	0.441	7.42	-10.25	0.71216
UpC	0.214	10.37	0.377	10.32	-10.25	0.71208

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
UpC	0.071	11.65	0.396	8.96	-10.05	0.71175
UpC	0.129	10.40	0.393	8.63	-10.30	0.71195
UpC	0.110	12.11	0.329	4.85	-10.10	0.71218
UpC	0.040	13.46	0.362	6.97	-10.33	0.71196
UpC	0.087	11.89	0.358	6.44	-10.03	0.71195
UpC	0.112	15.49	0.724	6.54	-9.88	0.71115
UpC	0.151	8.90	0.390	6.47	-10.15	0.71206
UpC	0.117	11.76	0.397	7.65	-10.23	0.71211
UpC	0.114	11.89	0.404	6.51	-10.21	0.71183
UpC	0.052	8.10	0.407	4.56	-9.99	0.71199
UpC	0.136	9.37	0.368	6.49	-10.12	0.71189
UpC	0.091	13.62	0.407	7.54	-10.11	0.71205
UpC	0.111	9.62	0.429	6.87	-10.19	0.71190
UpC	0.110	16.82	1.035	7.16	-10.14	0.71146
UpC	0.124	11.70	0.695	4.28	-9.64	0.71128
UpC	0.083	9.65	0.663	4.60	-9.50	0.71095
UpC	0.162	9.14	0.817	9.24	-9.84	0.71092
UpC	0.097	15.04	0.381	7.45	-10.33	0.71186
UpC	0.151	13.96	0.561	4.20	-9.39	0.71191
UpC	0.134	12.99	0.340	6.14	-9.48	0.71226
UpC	0.062	10.34	0.754	5.71	-9.71	0.71120
UpC	0.117	10.54	0.821	4.59	-9.27	0.71049
UpC	0.063	17.34	0.914	5.11	-9.84	0.71055
UpC	0.223	8.70	0.306	4.89	-9.42	0.71205
UpC	0.098	8.93	0.847	4.22	-9.62	0.71102
UpC	0.237	8.62	0.736	4.40	-9.49	0.71071
UpC	0.105	10.96	0.759	6.13	-9.32	0.71108
UpC	0.168	11.27	0.889	4.90	-8.94	0.71075
UpC	0.171	15.79	0.782	5.30	-9.39	0.71188
UpC	0.113	7.58	0.369	6.55	-8.74	0.71301
UpC	0.076	9.56	0.802	7.47	-9.37	0.71124
UpC	0.234	11.83	0.567	4.86	-9.71	0.71125

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ¹⁸O VPDB	⁸⁷Sr/⁸⁶Sr
UpC	0.267	8.45	0.775	8.55	-9.99	0.71096
UpC	0.112	16.56	0.452	5.28	-10.05	0.71176
UpC	0.250	12.36	0.449	6.26	-10.14	0.71193
UpC	0.143	11.38	0.409	7.73	-10.31	0.71199
UpC	0.116	10.38	0.886	4.44	-9.90	0.71063
UpC	0.148	10.04	0.411	4.82	-10.14	0.71198
UpC	0.103	13.29	0.687	4.85	-9.79	0.71097
UpC	0.108	12.16	0.565	10.18	-10.03	0.71155
Pot	0.133	9.69	0.398	6.57	-9.11	0.71092
Pot	0.232	8.06	0.374	5.50	-9.01	0.71086
Pot	0.139	9.04	0.381	5.15	-9.17	0.71079
Pot	0.248	6.86	0.381	5.49	-9.11	0.71009
Pot	0.113	11.02	0.425	5.26	-9.29	0.71095
Pot	0.170	9.85	0.373	6.17	-8.96	0.71062
Pot	0.221	9.70	0.350	7.25	-8.93	0.71099
Pot	0.193	8.35	0.375	7.21	-8.78	0.71087
Pot	0.103	10.42	0.410	6.52	-8.76	0.71097
Pot	0.176	14.61	0.343	4.36	-9.25	0.71082
Pot	0.106	7.41	0.374	5.98	-8.92	0.71114
Pot	0.062	7.03	0.513	7.71	-8.75	0.71071
Pot	0.103	8.51	0.522	5.57	-8.98	0.71103
Pot	0.165	7.60	0.402	5.31	-9.20	0.71095
Pot	0.195	10.71	0.403	7.45	-8.93	0.71103
Pot	0.315	10.22	0.360	5.35	-8.93	0.71093
Pot	0.133	5.77	0.437	4.59	-8.78	0.71088
Pot	0.332	10.52	0.420	6.27	-9.24	0.71091
Pot	0.089	6.72	0.453	7.67	-9.09	0.71099
Pot	0.063	8.76	0.398	5.73	-9.25	0.71094
Pot	0.096	11.82	0.407	6.76	-9.27	0.71092
Pot	0.287	6.77	0.318	5.33	-9.18	0.71091
Pot	0.175	9.60	0.407	4.60	-9.23	0.71091
Pot	0.147	6.16	0.348	4.86	-9.17	0.71089

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Pot	0.148	12.75	0.360	4.78	-9.24	0.71094
Pot	0.108	10.11	0.381	6.19	-9.24	0.71107
Pot	0.089	11.30	0.392	5.53	-9.38	0.71099
Pot	0.147	10.41	0.388	4.79	-9.08	0.71115
Pot	0.282	8.63	0.382	8.81	-8.79	0.71114
Pot	0.133	7.65	0.415	6.10	-9.11	0.71078
Pot	0.151	9.33	0.366	5.00	-8.87	0.71099
Pot	0.155	6.68	0.381	4.29	-9.10	0.71090
Pot	0.084	8.92	0.407	5.29	-8.84	0.71094
Pot	0.050	7.56	0.408	6.30	-9.02	0.71094
Pot	0.065	13.74	0.438	7.50	-8.71	0.71090
Pot	0.079	6.88	0.406	6.49	-9.10	0.71087
Pot	0.055	14.96	0.405	7.04	-8.98	0.71094
Pot	0.095	4.68	0.378	6.13	-9.25	0.71094
Pot	0.059	10.04	0.400	6.80	-9.08	0.71105
Pot	0.114	9.97	0.369	5.40	-9.27	0.71096
Pot	0.057	8.68	0.412	8.88	-9.32	0.71103
Pot	0.094	9.42	0.361	5.21	-9.23	0.71096
Pot	0.104	9.60	0.394	6.55	-9.25	0.71106
Pot	0.056	10.35	0.338	4.58	-9.15	0.71099
Pot	0.086	5.89	0.371	5.93	-9.31	0.71097
Pot	0.100	4.73	0.517	8.68	-9.18	0.71089
Pot	0.066	7.95	0.384	4.99	-9.17	0.71095
Pot	0.039	7.18	0.364	6.04	-9.03	0.71099
Pot	0.066	9.39	0.402	6.83	-9.26	0.71091
Pot	0.033	4.31	0.376	4.81	-8.88	0.71090
Pot	0.049	5.58	0.366	5.57	-9.18	0.71093
Pot	0.044	4.73	0.413	5.54	-8.96	0.71108
Pot	0.049	3.48	0.365	4.68	-8.97	0.71092
Pot	0.064	7.58	0.340	4.96	-9.00	0.71085
Pot	0.056	8.53	0.386	4.56	-9.06	0.71086
Pot	0.061	4.55	0.374	4.29	-9.10	0.71084

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Pot	0.065	7.95	0.392	5.29	-9.07	0.71093
Rap	0.098	9.22	0.561	21.03	-8.44	0.71669
Rap	0.172	11.89	0.907	14.86	-7.89	0.71472
Rap	0.137	16.98	0.841	23.56	-7.95	0.71549
Rap	0.079	11.39	0.487	12.01	-8.47	0.71712
Rap	0.082	11.14	0.561	13.72	-8.31	0.71681
Rap	0.104	5.88	0.484	24.34	-8.38	0.71650
Rap	0.101	5.62	0.514	23.16	-8.35	0.71629
Rap	0.139	7.28	0.604	16.79	-8.23	0.71674
Rap	0.126	9.16	0.525	18.61	-8.49	0.71607
Rap	0.132	13.53	0.863	13.92	-8.10	0.71534
Rap	0.071	4.46	0.525	25.59	-8.45	0.71639
Rap	0.151	10.23	0.511	16.66	-8.40	0.71671
Rap	0.155	6.67	0.488	13.67	-8.26	0.71646
Rap	0.089	6.38	0.546	11.03	-8.39	0.71617
Rap	0.158	4.05	0.516	20.35	-8.56	0.71639
Rap	0.266	4.74	0.441	15.94	-8.56	0.71630
Rap	0.112	4.59	0.523	16.68	-8.42	0.71662
Rap	0.166	9.56	0.477	22.61	-8.49	0.71621
Mat	0.065	6.01	0.967	21.53	-7.33	0.71203
Mat	0.110	21.52	0.841	22.90	-7.31	0.71228
Mat	0.068	20.32	0.937	18.87	-7.42	0.71266
Mat	0.102	19.50	0.767	22.61	-7.17	0.71271
Mat	0.125	9.78	0.869	15.64	-7.38	0.71230
Mat	0.093	8.58	0.763	18.27	-7.22	0.71219
Mat	0.088	16.39	0.972	23.29	-7.32	0.71254
Mat	0.100	19.58	0.792	29.62	-7.37	0.71277
Mat	0.125	21.23	0.775	24.25	-7.17	0.71235
Mat	0.177	22.59	0.812	22.48	-7.17	0.71250
Mat	0.180	11.95	0.866	24.11	-7.30	0.71273
Mat	0.132	23.18	0.879	20.89	-7.41	0.71227
Mat	0.125	15.92	0.801	21.49	-7.29	0.71206

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
Mat	0.160	22.37	0.848	33.56	-7.11	0.71287
Mat	0.107	16.17	0.791	21.38	-7.13	0.71248
Mat	0.097	21.55	0.769	22.13	-7.21	0.71209
Mat	0.093	11.79	0.855	12.15	-7.34	0.71215
Mat	0.127	15.59	0.869	18.62	-7.34	0.71273
Mat	0.189	15.39	0.768	29.24	-7.31	0.71194
Mat	0.081	22.80	0.774	26.02	-7.17	0.71243
Mat	0.111	18.05	0.844	18.36	-7.38	0.71196
Mat	0.152	16.49	0.903	21.36	-7.19	0.71157
Mat	0.355	15.93	0.680	19.96	-7.14	0.71174
Mat	0.123	20.25	0.707	18.00	-7.18	0.71239
Mat	0.099	6.93	0.994	15.96	-7.12	0.71140
Mat	0.109	22.13	0.762	19.05	-7.02	0.71218
Mat	0.102	7.66	0.866	15.72	-7.16	0.71218
Mat	0.269	14.96	0.602	19.74	-7.07	0.71214
Mat	0.074	18.78	0.993	18.16	-7.19	0.71153
Mat	0.131	23.40	0.762	20.95	-7.21	0.71162
Mat	0.078	15.84	0.894	26.63	-7.26	0.71279
Mat	0.068	14.26	1.136	21.78	-7.30	0.71229
Mat	0.076	15.20	0.963	28.50	-6.99	0.71297
Mat	0.111	16.64	1.000	22.60	-7.33	0.71127
Mat	0.077	11.68	1.120	19.26	-7.06	0.71157
Mat	0.158	12.78	1.039	17.48	-7.27	0.71135
Mat	0.160	10.46	0.906	15.99	-7.20	0.71127
Mat	0.138	23.98	0.882	19.27	-7.11	0.71104
Mat	0.106	15.37	1.091	22.63	-7.30	0.71151
Mat	0.117	21.77	0.815	20.58	-7.35	0.71222
Mat	0.165	24.70	0.878	24.20	-7.27	0.71171
Mat	0.223	24.34	0.957	21.11	-7.09	0.71222
Mat	0.121	23.83	0.792	20.91	-7.34	0.71220
Mat	0.128	16.34	0.981	25.41	-7.17	0.71138
Mat	0.142	24.50	0.820	23.91	-7.20	0.71207

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ¹⁸O VPDB	⁸⁷Sr/⁸⁶Sr
Mat	0.210	17.10	0.805	23.26	-7.21	0.71206
Mat	0.288	14.44	0.702	20.50	-7.12	0.71133
Mat	0.098	22.50	0.812	25.72	-7.24	0.71220
Mat	0.063	7.34	0.971	12.85	-7.22	0.71166
Mat	0.134	19.72	0.752	23.11	-6.99	0.71207
Mat	0.178	18.71	0.765	23.33	-7.14	0.71282
Mat	0.069	16.05	0.871	20.39	-7.34	0.71299
Mat	0.057	15.38	0.914	28.03	-7.31	0.71282
Mat	0.070	15.00	0.896	23.34	-7.40	0.71269
Mat	0.079	11.63	0.823	28.29	-7.39	0.71276
Mat	0.085	13.38	0.796	22.93	-7.17	0.71201
Mat	0.098	23.53	0.852	20.51	-7.19	0.71145
Mat	0.084	9.19	0.837	18.06	-7.52	0.71257
Mat	0.067	10.55	0.661	26.21	-7.35	0.71205
Roa	0.075	9.89	0.796	17.32	-6.67	0.71074
Roa	0.206	15.35	0.627	16.88	-7.08	0.71056
Roa	0.100	13.18	0.779	21.65	-7.09	0.71089
Roa	0.054	6.81	1.192	19.90	-6.73	0.71074
Roa	0.131	13.45	1.553	29.09	-6.75	0.71069
Roa	0.063	9.93	0.805	15.97	-7.45	0.71111
Roa	0.166	9.96	0.713	21.76	-7.42	0.71110
Roa	0.073	10.42	0.772	20.55	-7.33	0.71109
Roa	0.073	8.65	0.937	35.01	-7.35	0.71116
Roa	0.090	10.95	0.776	19.38	-7.40	0.71105
Roa	0.068	9.91	0.919	26.37	-7.56	0.71106
Roa	0.089	8.66	0.847	38.03	-7.27	0.71110
Roa	0.117	13.75	0.710	29.59	-6.98	0.71074
Roa	0.080	12.23	0.608	27.10	-7.60	0.71069
Roa	0.124	9.03	0.740	24.04	-7.33	0.71113
Roa	0.107	11.85	0.569	17.81	-6.98	0.71075
Roa	0.090	14.82	0.716	27.89	-6.89	0.71023
Roa	0.094	11.72	0.695	12.90	-7.36	0.71078

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
Roa	0.062	3.53	1.484	19.04	-7.02	0.71058
Roa	0.152	13.02	0.737	21.58	-7.05	0.71079
Roa	0.080	6.02	1.650	19.06	-6.78	0.71077
Roa	0.125	14.61	0.681	20.51	-6.52	0.71073
Roa	0.123	18.69	0.663	32.68	-6.92	0.71080
Roa	0.086	12.51	0.732	23.63	-6.77	0.71079
Roa	0.082	11.36	0.646	14.66	-7.19	0.71083
Roa	0.069	10.77	0.857	13.52	-7.19	0.71109
Roa	0.074	14.99	0.806	23.48	-7.16	0.71109
Roa	0.115	16.26	0.743	17.33	-7.38	0.71012
Roa	0.078	11.77	0.656	14.08	-6.48	0.71079
Roa	0.065	22.13	0.912	40.25	-6.89	0.71028
Roa	0.087	13.23	0.680	22.63	-6.67	0.71071
Roa	0.082	12.04	0.944	17.34	-6.75	0.71085
Roa	0.090	14.66	0.657	14.13	-6.68	0.71077
Roa	0.239	20.41	0.774	15.45	-6.95	0.71075
Roa	0.094	9.24	0.759	19.80	-6.90	0.71078
Roa	0.114	16.54	0.655	13.27	-6.95	0.71059
Roa	0.122	16.65	0.588	30.38	-7.28	0.71091
Roa	0.102	11.24	0.794	21.24	-6.98	0.71085
Roa	0.072	6.31	0.897	16.42	-6.55	0.71077
Roa	0.105	11.24	0.779	17.72	-6.87	0.71073
Roa	0.082	4.20	0.974	16.02	-6.90	0.71079
Roa	0.101	11.32	1.024	20.19	-6.88	0.71073
Roa	0.082	15.85	0.807	22.41	-6.84	0.71077
Roa	0.101	13.29	0.950	17.30	-6.81	0.71077
Roa	0.072	11.08	0.779	27.06	-7.41	0.71110
Roa	0.069	8.45	0.752	14.55	-6.62	0.71059
Roa	0.072	18.01	1.243	28.84	-6.59	0.71061
Roa	0.078	16.95	0.769	19.54	-7.04	0.71074
Roa	0.077	12.53	0.791	21.73	-6.52	0.71077
Roa	0.082	15.00	0.769	31.17	-6.70	0.71087

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Roa	0.097	14.16	0.783	19.28	-6.97	0.71062
Roa	0.095	12.52	0.942	21.72	-6.97	0.71064
Roa	0.108	11.93	0.638	22.00	-7.11	0.71089
Roa	0.168	15.17	0.601	14.93	-7.53	0.71113
Roa	0.091	15.16	0.791	19.23	-7.50	0.71116
Roa	0.090	9.52	0.711	17.97	-6.94	0.71077
StC	0.078	14.78	1.058	11.98	-6.62	0.70984
StC	0.064	11.93	1.145	21.99	-6.58	0.70981
StC	0.079	17.82	0.782	23.41	-6.37	0.70988
StC	0.069	14.69	1.347	21.51	-6.62	0.70980
StC	0.118	20.30	1.590	24.03	-6.35	0.70974
StC	0.179	9.82	1.681	23.27	-6.66	0.70979
StC	0.126	14.16	0.872	28.00	-6.58	0.70984
StC	0.096	14.37	1.799	28.22	-6.65	0.70980
StC	0.086	9.74	1.193	28.70	-6.57	0.70977
StC	0.146	16.39	1.238	19.61	-6.51	0.70980
StC	0.080	15.96	0.975	19.26	-6.61	0.70978
StC	0.210	7.17	1.070	24.64	-5.57	0.70981
StC	0.086	17.44	1.451	26.16	-6.55	0.70978
StC	0.155	12.71	1.297	17.42	-6.43	0.70979
StC	0.178	6.54	0.936	20.75	-5.94	0.70988
StC	0.110	10.37	1.797	20.43	-6.37	0.70979
StC	0.070	14.45	1.520	22.28	-6.61	0.70981
StC	0.074	19.89	1.067	18.40	-6.43	0.70984
StC	0.195	16.82	0.887	26.16	-6.72	0.70985
StC	0.107	18.16	1.367	17.23	-6.47	0.70981
StC	0.112	12.63	0.878	19.55	-6.21	0.70979
StC	0.152	14.68	1.462	23.59	-6.52	0.70979
StC	0.098	21.84	1.088	29.56	-6.57	0.70975
StC	0.083	17.31	1.813	21.20	-6.28	0.70978
StC	0.104	18.53	1.154	25.71	-6.63	0.70986
StC	0.146	15.55	1.456	21.00	-6.38	0.70976

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
StC	0.107	14.41	1.428	16.78	-6.66	0.70981
StC	0.070	13.98	1.084	24.56	-6.59	0.70982
StC	0.074	20.44	1.167	18.14	-6.73	0.70982
StC	0.133	26.12	0.907	21.10	-6.51	0.70984
StC	0.166	11.37	0.727	26.13	-6.85	0.70981
StC	0.163	15.26	1.006	14.00	-6.62	0.70980
StC	0.108	19.70	0.924	28.97	-6.64	0.70984
StC	0.158	10.44	1.091	15.38	-6.44	0.70982
StC	0.093	13.78	1.274	25.17	-6.65	0.70981
StC	0.113	15.36	1.109	19.38	-6.66	0.70979
StC	0.091	12.80	1.785	27.91	-6.32	0.70979
StC	0.117	11.34	0.987	17.57	-6.49	0.70983
StC	0.144	10.02	1.065	24.44	-6.63	0.70975
StC	0.091	13.75	1.215	30.39	-6.51	0.70982
StC	0.122	13.56	1.244	16.28	-6.63	0.70979
StC	0.133	23.61	1.097	28.81	-6.57	0.70983
StC	0.104	22.36	1.091	31.37	-6.59	0.70979
StC	0.157	14.66	1.295	28.92	-6.63	0.70982
StC	0.100	16.91	1.052	29.64	-6.58	0.70977
StC	0.089	12.42	1.294	22.90	-6.64	0.70978
StC	0.136	15.86	1.685	20.34	-6.51	0.70982
StC	0.068	16.30	1.172	22.67	-6.57	0.70986
StC	0.073	20.37	2.272	29.59	-6.37	0.70978
StC	0.157	11.75	1.062	33.73	-6.71	0.70981
StC	0.079	13.78	1.573	28.29	-6.73	0.70980
StC	0.255	27.59	0.729	14.14	-6.55	0.70981
StC	0.090	17.19	1.618	22.58	-6.76	0.70978
StC	0.148	15.81	1.582	19.27	-6.59	0.70982
StC	0.083	12.77	1.108	16.54	-6.27	0.70978
StC	0.226	11.07	0.906	12.95	-6.22	0.70978
StC	0.165	18.96	0.892	14.20	-6.49	0.70980
Alt	0.100	14.03	0.632	11.91	-5.72	0.71118

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
Alt	0.081	12.66	0.369	13.56	-5.52	0.71143
Alt	0.162	17.94	0.520	13.01	-5.59	0.71110
Alt	0.156	13.85	0.464	14.04	-5.98	0.71242
Alt	0.163	14.51	0.492	12.64	-5.77	0.71112
Alt	0.127	12.22	0.529	10.97	-5.86	0.71111
Alt	0.100	16.64	0.704	14.77	-5.70	0.71118
Alt	0.086	11.31	0.447	16.83	-5.75	0.71123
Alt	0.088	7.55	0.411	8.81	-5.60	0.71120
Alt	0.069	9.17	0.542	13.79	-5.66	0.71110
Alt	0.109	9.61	0.365	14.24	-5.51	0.71133
Alt	0.074	14.30	0.447	11.49	-5.75	0.71108
Alt	0.154	12.18	0.431	12.18	-5.95	0.71139
Alt	0.122	18.83	0.465	15.94	-6.18	0.71126
Alt	0.073	9.22	0.468	12.42	-5.74	0.71080
Alt	0.096	13.28	0.485	13.47	-5.76	0.71130
Alt	0.049	11.85	0.560	10.91	-5.60	0.71118
Alt	0.049	9.17	0.590	12.81	-5.84	0.71102
Alt	0.082	15.18	0.661	16.03	-5.71	0.71103
Alt	0.066	9.36	0.538	22.37	-5.57	0.71120
Alt	0.095	9.87	0.429	16.33	-5.77	0.71122
Alt	0.101	11.90	0.556	24.75	-5.69	0.71121
Alt	0.095	8.92	0.411	31.04	-5.27	0.71113
Alt	0.128	16.35	0.981	25.43	-5.83	0.71107
Alt	0.034	9.36	0.589	12.31	-5.84	0.71112
Alt	0.084	8.05	0.488	16.16	-5.65	0.71091
SJs	0.070	3.17	2.935	3.09	-2.19	0.70795
SJs	0.073	2.24	1.620	1.52	-2.85	0.70800
SJs	0.101	2.24	2.732	3.02	-1.84	0.70799
SJs	0.127	2.77	1.790	2.05	-2.50	0.70803
SJs	0.039	2.99	1.726	1.42	-2.52	0.70796
SJs	0.037	1.94	1.872	1.71	-1.93	0.70799
SJs	0.038	2.39	2.352	1.91	-2.24	0.70802

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ¹⁸O VPDB	⁸⁷Sr/⁸⁶Sr
SJs	0.134	3.76	1.728	1.45	-2.62	0.70801
SJs	0.052	2.48	2.548	2.24	-1.78	0.70799
SJs	0.118	2.55	2.635	1.99	-1.21	0.70801
SJs	0.207	3.42	2.259	2.20	-2.20	0.70799
SJs	0.081	4.33	2.314	2.03	-1.11	0.70799
SJs	0.086	2.57	1.884	1.68	-2.32	0.70797
SJs	0.087	4.48	2.566	3.09	-1.93	0.70799
SJs	0.066	3.53	2.165	2.11	-2.21	0.70800
SJs	0.045	4.27	1.566	1.61	-2.55	0.70802
SJs	0.052	3.46	2.368	2.52	-1.44	0.70799
SJs	0.049	2.14	1.580	1.75	-2.69	0.70797
SJs	0.093	3.42	2.322	2.09	-1.00	0.70799
SJs	0.115	2.09	2.042	2.02	-2.39	0.70800
SJs	0.075	1.99	1.715	1.81	-2.34	0.70804
SJs	0.094	3.14	2.363	2.22	-2.63	0.70796
SJs	0.230	2.57	2.330	1.93	-2.46	0.70799
SJs	0.184	2.80	1.520	1.48	-2.23	0.70800
SJs	0.078	3.26	2.480	2.36	-1.81	0.70799
SJs	0.209	1.74	1.963	1.81	-2.29	0.70797
SJs	0.054	3.99	2.202	1.99	-1.77	0.70798
SJs	0.094	2.29	1.485	1.59	-2.78	0.70800
SJs	0.057	4.10	2.467	2.60	-2.53	0.70799
SJs	0.057	2.40	1.837	2.14	-2.34	0.70801
SJs	0.098	2.23	1.837	1.76	-2.26	0.70801
SJs	0.097	2.30	2.093	1.82	-2.48	0.70796
SJs	0.063	3.49	1.540	1.44	-2.47	0.70803
SJs	0.128	3.23	2.473	2.17	-2.27	0.70800
SJs	0.090	3.02	1.659	1.92	-3.08	0.70793
SJs	0.111	2.30	2.439	2.15	-2.31	0.70801
SJs	0.123	2.11	1.819	1.46	-2.15	0.70801
SJs	0.073	2.56	2.446	1.87	-2.02	0.70798
SJs	0.085	1.96	2.235	2.10	-2.10	0.70800

Table A3.1 continued

River	Mg/Ca [mmol/mol]	Mn/Ca [μmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
SJs	0.034	2.05	2.099	1.30	-1.95	0.70797
SJs	0.050	1.96	1.961	1.89	-2.25	0.70798
SJs	0.062	3.03	1.868	2.02	-2.59	0.70797
SJs	0.137	2.76	2.226	2.07	-1.77	0.70799
SJs	0.099	2.78	1.836	1.78	-2.63	0.70796
SJs	0.160	2.04	2.137	2.34	-1.85	0.70798
SJs	0.094	2.14	2.495	2.45	-1.93	0.70799
SJs	0.059	2.62	1.565	2.04	-2.24	0.70796
SJs	0.098	2.25	2.496	1.67	-1.26	0.70800
SJs	0.142	3.29	1.944	2.07	-2.05	0.70799
SJs	0.109	2.79	2.292	2.14	-2.14	0.70800
SJs	0.051	2.02	1.798	1.91	-2.33	0.70801
SJs	0.073	3.12	2.213	1.99	-2.07	0.70799
SJs	0.177	4.06	2.179	2.50	-1.33	0.70797
SJs	0.094	3.06	2.514	2.40	-2.14	0.70799
SJs	0.074	2.19	2.900	2.52	-1.77	0.70798
SJs	0.151	2.08	2.350	2.42	-2.46	0.70799
SJs	0.062	2.09	1.640	1.35	-2.46	0.70798
SJs	0.051	2.44	1.561	1.40	-2.64	0.70800
SJs	0.050	3.73	2.469	2.04	-2.17	0.70799

Table A3.2 Water samples collected from rivers in 2004 and used for analyses reported in Chapter 3. Each row reports corrected elemental and isotopic ratios obtained from samples collected at one site along the river. River codes. Mir: Miramichi River, NB. StL: St. Lawrence River, QB. Shu: Shubenacadie River, NS. SJn: St. John River, NB. Ann: Annapolis River, NS. Ken: Kennebec River, ME. Mer: Merrimack River, MA. Con: Connecticut River, CT. Hud: Hudson River, NY. Mat: Mattaponi River, VA. Pam: Pamunkey River, VA. SJs: St. Johns River, Florida

River	Site Name	Mg/Ca [mol/mol]	Mn/Ca [mmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [mmol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
Mir	Quarryville	0.192	4.34	3.08	0.631	-9.88	0.71084
Mir	Blackville	0.226	2.70	4.43	0.444	-10.09	0.71087
Mir	Upper Blackville	0.229	1.05	3.90	0.242	-10.18	0.71108
Mir	Red Bank	0.502	5.87	8.09	0.452	-10.39	0.71071
Mir	Sevogle	0.169	0.86	1.07	0.145	-11.01	0.71318
SJn	Woodstock Road	0.198	0.61	2.84	0.165	-9.91	0.71063
SJn	Grand Lake	0.249	0.24	4.57	0.608	-9.99	0.71069
SJn	Washademoak Lake	0.191	0.85	3.49	0.817	-9.03	0.71037
SJn	Kennebecasis River	0.187	1.45	3.51	0.837	-7.78	0.70940
Shu	Enfield	0.214	1.09	1.76	0.470	-7.11	0.71090
Shu	Elmsdale	0.213	2.60	1.75	0.442	-7.26	0.71089
Shu	Milford	0.141	3.38	2.33	0.207	-7.04	0.70910
Shu	Shubenacadie Town	0.149	4.15	2.55	0.080	-7.07	0.70862
Ann	Middleton	0.214	1.81	1.87	0.234	-8.57	0.71151
Ann	Kingston	0.215	2.47	1.19	0.293	-8.82	0.71177
Ann	Auburn	0.223	2.39	1.36	0.286	-8.73	0.71167
StL	Trois-Rivieres	0.402	0.22	2.23	0.205	-7.87	0.70970
StL	Montreal	0.400	0.06	2.16	0.183	-7.03	0.70938
StL	Kahnawake	0.407	0.02	2.18	0.184	-6.79	0.70936
StL	Saint-Francois	0.396	0.02	2.11	0.172	-6.60	0.70936
Ken	Richmond	0.326	0.51	2.08	0.182	-8.71	0.71359
Ken	Hallowell	0.318	0.73	2.07	0.207	-9.09	0.71359
Ken	Sidney	0.314	1.08	1.89	0.209	-9.26	0.71391
Ken	Waterville	0.339	1.22	1.99	0.298	-9.36	0.71403
Mer	Newburyport	0.377	1.94	2.78	0.361	-7.37	0.71417

Table A3.2 continued

River	Site Name	Mg/Ca [mol/mol]	Mn/Ca [mmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [mmol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
Mer	Lawrence	0.291	4.77	2.88	0.430	-7.42	0.71451
Mer	Lowell	0.282	7.09	3.03	0.472	-7.42	0.71491
Mer	Manchester	0.303	6.17	3.24	0.450	-7.52	0.71367
Mer	Litchfield	0.308	15.73	3.43	0.553	-7.60	0.71396
Con	Brattleboro	0.214	1.38	1.97	0.245	-8.87	0.71308
Con	Turners Falls	0.217	0.32	1.99	0.251	-8.44	0.71341
Con	Holyoke	0.230	2.47	2.02	0.307	-8.55	0.71374
Con	Windsor Locks	0.247	1.91	2.23	0.373	-8.19	0.71332
Con	Haddam Meadows	0.264	0.78	2.14	0.396	-7.91	0.71363
Hud	Newburgh	0.286	0.44	2.01	0.207	-8.08	0.71145
Hud	Kingston	0.274	0.13	2.10	0.205	-8.61	0.71116
Hud	Catskill	0.256	0.17	1.92	0.220	-8.81	0.71157
Hud	Coxsackie	0.271	0.23	1.88	0.209	-8.76	0.71127
Hud	Troy	0.272	0.58	1.97	0.207	-8.93	0.71077
Mat	Water Fence	0.708	8.14	2.95	1.028	-7.20	0.71179
Mat	Melrose	0.523	15.62	2.62	1.502	-6.78	0.71395
Mat	Rainbow Acres	0.505	21.99	2.55	1.529	-6.62	0.71296
Mat	Walkerton	0.736	16.14	2.85	2.106	-5.92	0.71342
Mat	Aylett	0.758	26.83	3.05	2.847	-6.47	0.71390
Pam	Sweet Hall	0.522	7.85	2.58	1.061	-7.78	0.71169
Pam	Riverview	0.475	8.73	2.40	0.983	-6.65	0.71168
Pam	Lester Manor	0.487	9.34	3.11	1.029	-8.00	0.71174
Pam	Mantapike	0.599	5.71	2.59	1.470	-5.34	0.71297
Pam	New Castle	0.601	8.71	2.55	1.437	-5.30	0.71292
SJs	Palatka	0.520	0.24	6.42	0.107	-1.95	0.70801
SJs	Lake George	0.625	0.94	8.28	0.127	-2.08	0.70799
SJs	DeBary	0.502	0.71	7.67	0.178	-3.40	0.70810
SJs	Lake Harney	0.467	1.59	4.19	0.197	-3.55	0.70851
SJs	Lake Poinsett	0.387	0.55	12.12	0.186	-2.01	0.70801

APPENDIX 4

ANALYTICAL DATA FOR SAMPLES USED IN CHAPTER 4

Table A4.1 Immature American shad collected along the coast of Maine in trawl surveys conducted in the spring of 2005 and used for analyses reported in Chapter 4. Each row reports corrected elemental and isotopic ratios obtained from the otoliths of one individual fish.

Trawl Date	Latitude (N)	Longitude (W)	Sr/Ca [mmol/mol]	Ba/Ca [μ mol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
5/3/2005	42° 57' 24"	70° 28' 09"	0.452	5.29	-9.79	0.70995
5/3/2005	42° 57' 24"	70° 28' 09"	0.555	4.79	-9.14	0.70990
5/3/2005	42° 57' 24"	70° 28' 09"	0.855	11.44	-8.67	0.71092
5/3/2005	42° 58' 08"	70° 23' 58"	0.528	5.25	-9.71	0.71050
5/3/2005	42° 58' 08"	70° 23' 58"	0.446	6.66	-10.82	0.71121
5/3/2005	42° 58' 08"	70° 23' 58"	0.458	6.32	-10.13	0.71108
5/3/2005	43° 02' 13"	70° 33' 31"	0.868	15.18	-10.30	0.70895
5/3/2005	43° 02' 13"	70° 33' 31"	0.503	3.44	-10.89	0.71038
5/4/2005	43° 04' 29"	70° 17' 55"	0.870	14.98	-9.91	0.70905
5/4/2005	43° 04' 29"	70° 17' 55"	0.481	7.15	-10.51	0.71102
5/4/2005	43° 04' 29"	70° 17' 55"	0.827	13.57	-11.03	0.71024
5/4/2005	43° 05' 16"	70° 29' 16"	0.465	3.94	-9.15	0.70972
5/4/2005	43° 05' 16"	70° 29' 16"	0.574	4.62	-10.99	0.71036
5/4/2005	43° 09' 17"	70° 22' 34"	0.486	4.53	-8.79	0.70978
5/4/2005	43° 10' 19"	70° 23' 59"	0.998	7.83	-8.36	0.71314
5/4/2005	43° 10' 19"	70° 23' 59"	0.649	9.21	-10.74	0.71041
5/4/2005	43° 10' 19"	70° 23' 59"	0.456	5.85	-9.33	0.71102
5/4/2005	43° 10' 19"	70° 23' 59"	0.910	9.23	-9.62	0.71089
5/4/2005	43° 10' 19"	70° 23' 59"	0.500	3.94	-11.17	0.71039
5/5/2005	43° 17' 05"	70° 30' 16"	0.483	4.85	-10.23	0.71059
5/5/2005	43° 17' 05"	70° 30' 16"	0.441	6.63	-9.82	0.71106
5/5/2005	43° 17' 05"	70° 30' 16"	0.497	5.67	-9.39	0.71005
5/5/2005	43° 17' 05"	70° 30' 16"	0.464	6.39	-10.79	0.71105
5/5/2005	43° 17' 05"	70° 30' 16"	0.812	5.49	-11.34	0.71061
5/5/2005	43° 17' 05"	70° 30' 16"	0.480	3.86	-8.79	0.71010

Table A4.1 continued

Trawl Date	Latitude (N)	Longitude (W)	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
5/5/2005	43° 23' 44"	69° 07' 11"	0.481	4.53	-9.12	0.70978
5/5/2005	43° 23' 44"	69° 07' 11"	0.463	4.42	-9.36	0.70972
5/5/2005	43° 23' 44"	69° 07' 11"	0.479	8.28	-9.43	0.71116
5/5/2005	43° 23' 44"	69° 07' 11"	0.478	6.88	-10.30	0.71102
5/6/2005	43° 26' 02"	70° 16' 43"	0.550	5.52	-11.39	0.71071
5/10/2005	43° 25' 08"	69° 56' 24"	0.485	8.28	-9.69	0.71076
5/10/2005	43° 28' 49"	69° 55' 59"	0.797	13.29	-10.22	0.70898
5/11/2005	43° 36' 43"	69° 51' 45"	0.442	4.67	-9.43	0.70974
5/11/2005	43° 36' 43"	69° 51' 45"	0.606	3.76	-11.80	0.71022
5/11/2005	43° 36' 43"	69° 51' 45"	0.463	3.48	-11.45	0.71040
5/11/2005	43° 36' 43"	69° 51' 45"	0.479	6.21	-11.00	0.71058
5/11/2005	43° 40' 32"	69° 58' 25"	0.656	11.06	-10.46	0.71038
5/11/2005	43° 40' 32"	69° 58' 25"	0.494	8.67	-9.79	0.71115
5/12/2005	43° 44' 36"	69° 39' 02"	1.887	19.73	-5.18	0.71197
5/12/2005	43° 44' 36"	69° 39' 02"	1.658	5.27	-3.76	0.71068
5/12/2005	43° 44' 36"	69° 39' 02"	0.723	13.60	-10.17	0.70891
5/12/2005	43° 44' 36"	69° 39' 02"	0.900	7.13	-11.23	0.71065
5/12/2005	43° 44' 36"	69° 39' 02"	0.463	7.04	-9.97	0.71091
5/12/2005	43° 44' 36"	69° 39' 02"	0.486	7.25	-10.68	0.71101
5/12/2005	43° 44' 36"	69° 39' 02"	0.396	5.44	-10.41	0.71105
5/12/2005	43° 44' 36"	69° 39' 02"	0.486	5.34	-9.06	0.70965
5/12/2005	43° 44' 36"	69° 39' 02"	0.449	4.71	-9.01	0.70972
5/12/2005	43° 44' 36"	69° 39' 02"	0.475	4.86	-8.48	0.70996
5/12/2005	43° 44' 36"	69° 39' 02"	0.555	4.19	-11.71	0.71043
5/12/2005	43° 44' 36"	69° 39' 02"	0.483	5.64	-9.24	0.70975
5/12/2005	43° 44' 36"	69° 39' 02"	0.477	5.96	-9.01	0.70969
5/12/2005	43° 44' 36"	69° 39' 02"	0.513	5.10	-8.43	0.70996
5/12/2005	43° 44' 36"	69° 39' 02"	0.440	4.22	-9.88	0.70967
5/12/2005	43° 44' 36"	69° 39' 02"	0.517	6.12	-9.11	0.70979
5/12/2005	43° 44' 36"	69° 39' 02"	0.450	4.02	-9.51	0.70970
5/12/2005	43° 44' 36"	69° 39' 02"	0.495	6.89	-10.31	0.71110
5/12/2005	43° 44' 36"	69° 39' 02"	0.484	5.26	-9.16	0.71031

Table A4.1 continued

Trawl Date	Latitude (N)	Longitude (W)	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ¹⁸O VPDB	⁸⁷Sr/⁸⁶Sr
5/12/2005	43° 44' 36"	69° 39' 02"	0.529	5.66	-7.76	0.70964
5/12/2005	43° 44' 36"	69° 39' 02"	0.531	6.45	-9.81	0.71002
5/12/2005	43° 44' 36"	69° 39' 02"	0.966	17.47	-10.10	0.71091
5/12/2005	43° 44' 36"	69° 39' 02"	0.539	5.12	-8.57	0.70973
5/12/2005	43° 44' 36"	69° 39' 02"	0.482	5.76	-9.77	0.70972
5/12/2005	43° 44' 36"	69° 39' 02"	0.487	5.95	-9.94	0.70972
5/12/2005	43° 44' 36"	69° 39' 02"	0.454	7.64	-10.36	0.71095
5/12/2005	43° 44' 36"	69° 39' 02"	0.514	7.08	-9.61	0.71014
5/12/2005	43° 44' 36"	69° 39' 02"	0.502	5.63	-9.81	0.71009
5/12/2005	43° 44' 36"	69° 39' 02"	0.471	5.48	-9.64	0.71004
5/12/2005	43° 44' 36"	69° 39' 02"	0.459	2.94	-11.87	0.71039
5/12/2005	43° 44' 36"	69° 39' 02"	0.442	3.38	-9.43	0.70971
5/12/2005	43° 44' 36"	69° 39' 02"	0.499	4.07	-11.42	0.71040
5/12/2005	43° 44' 36"	69° 39' 02"	0.460	4.67	-9.28	0.71025
5/12/2005	43° 44' 36"	69° 39' 02"	0.435	7.42	-9.45	0.71120
5/12/2005	43° 44' 36"	69° 39' 02"	0.481	4.93	-9.52	0.70961
5/12/2005	43° 44' 36"	69° 39' 02"	0.548	4.31	-9.16	0.70994
5/12/2005	43° 44' 36"	69° 39' 02"	0.536	3.91	-11.54	0.71036
5/12/2005	43° 44' 36"	69° 39' 02"	0.555	3.18	-8.16	0.71008
5/12/2005	43° 44' 36"	69° 39' 02"	0.797	15.51	-11.08	0.71018
5/12/2005	43° 44' 36"	69° 39' 02"	0.491	4.18	-8.46	0.70977
5/12/2005	43° 44' 36"	69° 39' 02"	0.487	4.45	-9.47	0.71045
5/12/2005	43° 44' 36"	69° 39' 02"	0.437	3.09	-9.51	0.70980
5/12/2005	43° 44' 36"	69° 39' 02"	0.439	5.18	-9.89	0.71010
5/12/2005	43° 44' 36"	69° 39' 02"	0.457	6.46	-10.30	0.71088
5/12/2005	43° 44' 36"	69° 39' 02"	0.439	4.87	-9.79	0.70953
5/12/2005	43° 44' 36"	69° 39' 02"	2.056	13.46	-7.82	0.71061
5/12/2005	43° 44' 36"	69° 39' 02"	0.493	6.76	-9.91	0.71046
5/12/2005	43° 44' 36"	69° 39' 02"	0.514	4.77	-7.05	0.71005
5/12/2005	43° 44' 36"	69° 39' 02"	0.504	4.89	-9.13	0.71032
5/12/2005	43° 44' 36"	69° 39' 02"	0.452	4.88	-9.12	0.70998
5/12/2005	43° 44' 36"	69° 39' 02"	0.532	3.98	-9.26	0.70984

Table A4.1 continued

Trawl Date	Latitude (N)	Longitude (W)	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ¹⁸O VPDB	⁸⁷Sr/⁸⁶Sr
5/12/2005	43° 44' 36"	69° 39' 02"	0.463	5.06	-9.42	0.71005
5/12/2005	43° 44' 36"	69° 39' 02"	0.528	5.95	-9.94	0.71055
5/12/2005	43° 44' 36"	69° 39' 02"	0.507	4.95	-9.51	0.71022
5/12/2005	43° 44' 36"	69° 39' 02"	0.469	6.20	-9.07	0.70987
5/12/2005	43° 44' 36"	69° 39' 02"	0.515	5.18	-9.74	0.71046
5/12/2005	43° 44' 36"	69° 39' 02"	0.506	5.79	-9.72	0.70983
5/12/2005	43° 44' 36"	69° 39' 02"	0.526	4.42	-9.39	0.71000
5/12/2005	43° 44' 36"	69° 39' 02"	0.441	5.35	-9.97	0.70971
5/12/2005	43° 44' 36"	69° 39' 02"	0.637	5.36	-9.95	0.70965
5/12/2005	43° 44' 36"	69° 39' 02"	0.446	5.38	-9.00	0.71042
5/12/2005	43° 44' 36"	69° 39' 02"	0.494	5.27	-10.00	0.71091
5/12/2005	43° 44' 36"	69° 39' 02"	0.475	7.81	-10.71	0.71079
5/12/2005	43° 44' 36"	69° 39' 02"	0.488	4.85	-9.20	0.70970
5/12/2005	43° 44' 36"	69° 39' 02"	0.446	4.08	-9.47	0.71023
5/12/2005	43° 44' 36"	69° 39' 02"	0.452	4.93	-10.97	0.71116
5/12/2005	43° 44' 36"	69° 39' 02"	0.469	6.37	-10.82	0.71029
5/12/2005	43° 44' 36"	69° 39' 02"	0.813	15.05	-11.14	0.71025
5/12/2005	43° 44' 36"	69° 39' 02"	0.635	8.07	-11.50	0.71040
5/12/2005	43° 44' 36"	69° 39' 02"	0.517	3.01	-11.33	0.71039
5/12/2005	43° 44' 36"	69° 39' 02"	0.481	5.76	-9.28	0.70963
5/12/2005	43° 44' 36"	69° 39' 02"	0.994	17.92	-9.85	0.71091
5/12/2005	43° 44' 36"	69° 39' 02"	0.442	4.25	-8.26	0.70998
5/12/2005	43° 44' 36"	69° 39' 02"	0.515	3.80	-9.06	0.70970
5/12/2005	43° 47' 46"	69° 42' 01"	1.982	21.23	-3.93	0.71290
5/12/2005	43° 47' 46"	69° 42' 01"	0.445	6.10	-10.40	0.71087
5/12/2005	43° 47' 46"	69° 42' 01"	0.489	5.64	-8.63	0.70968
5/13/2005	43° 46' 25"	69° 27' 46"	0.521	2.95	-11.43	0.71032
5/13/2005	43° 46' 25"	69° 27' 46"	0.462	4.65	-9.10	0.70973
5/13/2005	43° 46' 25"	69° 27' 46"	0.488	3.96	-9.15	0.71011
5/13/2005	43° 46' 25"	69° 27' 46"	0.518	3.61	-11.41	0.71034
5/13/2005	43° 48' 26"	69° 21' 23"	0.692	8.93	-10.52	0.70898
5/13/2005	43° 48' 26"	69° 21' 23"	0.533	5.34	-10.67	0.71101

Table A4.1 continued

Trawl Date	Latitude (N)	Longitude (W)	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ¹⁸O VPDB	⁸⁷Sr/⁸⁶Sr
5/13/2005	43° 48' 26"	69° 21' 23"	0.503	5.84	-9.52	0.71057
5/13/2005	43° 48' 26"	69° 21' 23"	0.522	5.76	-11.09	0.70965
5/13/2005	43° 48' 26"	69° 21' 23"	0.679	7.16	-9.95	0.71049
5/13/2005	43° 48' 26"	69° 21' 23"	0.522	5.40	-11.81	0.71058
5/13/2005	43° 48' 26"	69° 21' 23"	0.543	4.99	-9.46	0.71045
5/13/2005	43° 48' 26"	69° 21' 23"	0.426	5.44	-10.08	0.71094
5/13/2005	43° 48' 26"	69° 21' 23"	0.527	3.23	-11.93	0.71043
5/13/2005	43° 48' 26"	69° 21' 23"	0.492	4.74	-9.51	0.70965
5/13/2005	43° 50' 30"	69° 28' 11"	0.514	4.30	-11.44	0.71039
5/13/2005	43° 50' 30"	69° 28' 11"	0.529	5.06	-9.77	0.70957
5/13/2005	43° 50' 30"	69° 28' 11"	0.668	8.25	-11.82	0.71046
5/14/2005	43° 40' 44"	69° 25' 10"	0.498	4.70	-9.50	0.71052
5/14/2005	43° 40' 44"	69° 25' 10"	0.474	5.44	-9.67	0.71104
5/14/2005	43° 40' 44"	69° 25' 10"	0.506	5.35	-8.51	0.71012
5/14/2005	43° 40' 44"	69° 25' 10"	0.461	3.87	-8.32	0.70991
5/14/2005	43° 40' 44"	69° 25' 10"	0.519	4.08	-8.48	0.71050
5/14/2005	43° 41' 48"	69° 30' 42"	0.483	3.28	-9.93	0.71041
5/14/2005	43° 41' 48"	69° 30' 42"	0.441	6.54	-8.56	0.71020
5/14/2005	43° 41' 48"	69° 30' 42"	0.527	4.22	-9.18	0.71037
5/14/2005	43° 41' 48"	69° 30' 42"	0.469	4.08	-9.10	0.70992
5/14/2005	43° 41' 48"	69° 30' 42"	0.460	7.40	-10.16	0.71092
5/14/2005	43° 41' 48"	69° 30' 42"	0.585	6.13	-9.66	0.71035
5/16/2005	43° 52' 41"	69° 04' 48"	0.520	4.93	-9.54	0.70968
5/16/2005	43° 53' 12"	69° 13' 49"	0.473	4.97	-9.10	0.71009
5/16/2005	43° 53' 12"	69° 13' 49"	0.490	3.50	-11.75	0.71045
5/16/2005	43° 53' 12"	69° 13' 49"	0.458	3.89	-9.29	0.70963
5/16/2005	43° 53' 12"	69° 13' 49"	0.704	4.67	-10.36	0.71026
5/16/2005	43° 53' 12"	69° 13' 49"	0.459	5.95	-10.23	0.71097
5/17/2005	44° 08' 02"	69° 01' 14"	0.461	4.68	-9.31	0.70977
5/17/2005	44° 08' 52"	69° 04' 17"	0.621	3.83	-8.64	0.71025
5/17/2005	44° 08' 52"	69° 04' 17"	1.070	10.92	-9.83	0.71107
5/17/2005	44° 08' 52"	69° 04' 17"	0.504	6.08	-8.96	0.70978

Table A4.1 continued

Trawl Date	Latitude (N)	Longitude (W)	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ¹⁸O VPDB	⁸⁷Sr/⁸⁶Sr
5/18/2005	43° 35' 07"	69° 04' 26"	0.479	5.61	-9.38	0.71005
5/18/2005	43° 35' 07"	69° 04' 26"	0.523	5.35	-11.82	0.71044
5/18/2005	43° 36' 29"	68° 56' 12"	0.891	13.81	-10.87	0.71003
5/18/2005	43° 36' 29"	68° 56' 12"	0.451	4.69	-9.08	0.71031
5/18/2005	43° 46' 01"	69° 07' 11"	0.784	11.91	-9.89	0.70914
5/18/2005	43° 46' 01"	69° 07' 11"	0.468	5.07	-9.24	0.70976
5/18/2005	43° 46' 01"	69° 07' 11"	0.494	5.14	-10.16	0.71016
5/18/2005	43° 46' 01"	69° 07' 11"	0.549	7.58	-10.45	0.71098
5/18/2005	43° 46' 01"	69° 07' 11"	0.822	15.77	-11.17	0.71018
5/18/2005	43° 46' 12"	69° 13' 13"	0.491	6.57	-9.69	0.71087
5/18/2005	43° 46' 12"	69° 13' 13"	0.977	16.17	-10.34	0.71088
5/18/2005	43° 46' 12"	69° 13' 13"	0.624	4.08	-11.61	0.71019
5/19/2005	43° 46' 52"	68° 38' 05"	0.479	4.44	-8.99	0.70971
5/19/2005	43° 46' 52"	68° 38' 05"	1.824	6.84	-7.13	0.70920
5/19/2005	43° 46' 52"	68° 38' 05"	0.556	7.52	-8.88	0.70942
5/19/2005	43° 46' 52"	68° 38' 05"	0.484	4.39	-9.23	0.70969
5/20/2005	43° 55' 04"	68° 37' 20"	0.453	6.37	-9.50	0.71107
5/20/2005	43° 55' 04"	68° 37' 20"	0.424	3.65	-8.80	0.70978
5/20/2005	43° 55' 04"	68° 37' 20"	0.484	4.30	-9.58	0.70975
5/20/2005	43° 55' 13"	68° 47' 09"	0.559	4.28	-11.79	0.71027
5/20/2005	43° 55' 13"	68° 47' 09"	0.485	3.00	-11.82	0.71040
5/20/2005	43° 55' 13"	68° 47' 09"	1.017	24.56	-10.25	0.70953
5/20/2005	43° 55' 13"	68° 47' 09"	0.524	9.02	-11.27	0.71065
5/20/2005	43° 55' 13"	68° 47' 09"	0.470	5.51	-8.22	0.71116
5/20/2005	44° 00' 33"	68° 52' 26"	0.480	11.21	-11.25	0.71103
5/20/2005	44° 00' 33"	68° 52' 26"	0.449	6.43	-10.75	0.71096
5/20/2005	44° 00' 33"	68° 52' 26"	0.630	7.68	-10.31	0.71244
5/20/2005	44° 08' 41"	68° 45' 41"	0.832	18.67	-10.04	0.70888
5/20/2005	44° 08' 41"	68° 45' 41"	0.551	7.51	-9.47	0.71045
5/20/2005	44° 08' 41"	68° 45' 41"	0.476	4.81	-9.45	0.70987
5/20/2005	44° 08' 41"	68° 45' 41"	0.460	4.84	-9.55	0.70969
5/20/2005	44° 08' 41"	68° 45' 41"	0.552	6.38	-11.58	0.71037

Table A4.1 continued

Trawl Date	Latitude (N)	Longitude (W)	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
5/20/2005	44° 08' 41"	68° 45' 41"	0.539	5.62	-10.71	0.71186
5/30/2005	44° 01' 43"	68° 24' 48"	0.469	8.25	-11.54	0.71073
5/31/2005	43° 55' 17"	68° 17' 29"	0.491	4.80	-9.30	0.70996
5/31/2005	43° 57' 54"	68° 16' 46"	0.482	6.30	-10.49	0.71108
5/31/2005	43° 58' 02"	68° 19' 25"	0.428	4.20	-9.26	0.70987
6/1/2005	44° 08' 43"	68° 15' 35"	0.557	4.88	-11.70	0.71033
6/1/2005	44° 08' 43"	68° 15' 35"	0.538	4.03	-11.11	0.71033
6/1/2005	44° 08' 43"	68° 15' 35"	0.447	4.28	-8.73	0.70964
6/1/2005	44° 08' 43"	68° 15' 35"	0.635	6.07	-10.31	0.71037
6/1/2005	44° 18' 06"	68° 31' 10"	0.472	4.68	-9.03	0.70967
6/3/2005	44° 01' 46"	68° 00' 13"	0.739	4.85	-11.14	0.71019
6/3/2005	44° 15' 42"	68° 06' 00"	0.476	6.13	-9.78	0.71079
6/3/2005	44° 15' 42"	68° 06' 00"	0.585	6.30	-9.16	0.70993
6/3/2005	44° 15' 42"	68° 06' 00"	0.435	3.89	-8.87	0.70969
6/3/2005	44° 21' 10"	68° 10' 02"	0.409	5.99	-10.10	0.71087
6/6/2005	44° 14' 41"	67° 53' 00"	0.538	5.38	-9.05	0.70990
6/6/2005	44° 14' 41"	67° 53' 00"	0.562	4.87	-9.16	0.70979
6/6/2005	44° 14' 41"	67° 53' 00"	0.495	3.96	-8.97	0.70982
6/6/2005	44° 14' 41"	67° 53' 00"	0.517	3.70	-9.17	0.71032
6/6/2005	44° 14' 41"	67° 53' 00"	0.576	5.33	-9.09	0.71054
6/6/2005	44° 14' 41"	67° 53' 00"	0.447	5.78	-10.51	0.71097
6/7/2005	44° 17' 21"	67° 44' 04"	0.786	18.48	-9.81	0.70877
6/7/2005	44° 17' 21"	67° 44' 04"	0.455	6.21	-9.44	0.71029
6/7/2005	44° 21' 32"	67° 41' 43"	0.448	4.05	-9.14	0.70983
6/7/2005	44° 21' 32"	67° 41' 43"	0.519	7.23	-11.79	0.71091
6/7/2005	44° 21' 32"	67° 41' 43"	0.549	5.08	-10.13	0.71060
6/7/2005	44° 21' 32"	67° 41' 43"	0.526	6.87	-10.11	0.71073
6/7/2005	44° 21' 32"	67° 41' 43"	0.463	4.77	-9.04	0.70981
6/7/2005	44° 21' 32"	67° 41' 43"	0.487	10.42	-9.06	0.71088
6/7/2005	44° 21' 32"	67° 41' 43"	0.440	5.87	-10.25	0.71106
6/7/2005	44° 21' 32"	67° 41' 43"	0.503	5.26	-9.06	0.71007
6/7/2005	44° 27' 50"	67° 43' 24"	0.456	5.40	-9.98	0.71125

Table A4.1 continued

Trawl Date	Latitude (N)	Longitude (W)	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ¹⁸O VPDB	⁸⁷Sr/⁸⁶Sr
6/7/2005	44° 27' 50"	67° 43' 24"	0.475	7.92	-10.47	0.71087
6/7/2005	44° 27' 50"	67° 43' 24"	0.416	6.09	-9.69	0.71113
6/7/2005	44° 27' 50"	67° 43' 24"	0.460	6.91	-10.43	0.71094

Table A4.2 Immature American shad collected in Minas Basin in a commercial herring weir at Five Islands, Nova Scotia in the summer of 2005 and used for analyses reported in Chapter 4. Each row reports corrected elemental and isotopic ratios obtained from the otoliths of one individual fish.

Capture Date	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
6/27/2005	0.561	5.07	-9.01	0.70995
6/27/2005	0.503	7.08	-9.40	0.71041
6/27/2005	0.489	6.54	-11.56	0.71103
6/27/2005	0.440	3.96	-9.07	0.70989
6/27/2005	0.607	7.23	-9.84	0.71035
6/27/2005	0.420	7.68	-11.07	0.71117
6/27/2005	0.520	5.86	-9.55	0.71049
6/27/2005	0.484	6.01	-9.48	0.70971
6/27/2005	0.457	4.18	-9.21	0.70975
6/27/2005	0.515	10.51	-10.07	0.71080
6/27/2005	0.608	7.68	-9.61	0.71057
6/27/2005	0.450	5.99	-9.53	0.71092
6/28/2005	0.490	6.82	-10.10	0.71048
6/28/2005	0.494	6.21	-10.91	0.71075
6/28/2005	0.479	5.98	-9.28	0.70996
6/29/2005	0.549	7.07	-9.30	0.71017
6/29/2005	0.478	5.74	-10.33	0.71117
6/29/2005	0.461	5.81	-9.17	0.70975
6/29/2005	0.530	9.57	-10.19	0.71108
6/29/2005	0.513	7.74	-9.74	0.71093
6/29/2005	0.618	4.81	-8.97	0.70986
6/29/2005	0.485	6.97	-9.11	0.71107
6/29/2005	0.505	7.37	-9.49	0.70985
6/29/2005	0.516	7.14	-10.46	0.71086
6/29/2005	0.665	7.25	-8.56	0.70987
6/29/2005	0.539	4.07	-9.85	0.71046
6/29/2005	0.511	5.36	-9.35	0.70982
6/29/2005	0.515	7.19	-11.60	0.71114
6/29/2005	0.489	4.33	-9.42	0.71020

Table A4.2 continued

Capture Date	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
6/29/2005	0.496	4.67	-8.68	0.70999
6/30/2005	0.566	6.55	-9.72	0.71040
6/30/2005	0.516	5.16	-8.20	0.70947
7/1/2005	0.482	7.82	-9.55	0.71062
7/1/2005	0.460	7.38	-11.19	0.71123
7/1/2005	0.499	3.61	-9.36	0.70968
7/1/2005	0.639	6.95	-9.07	0.70968
7/1/2005	0.548	5.69	-9.55	0.71039
7/2/2005	0.505	5.62	-8.99	0.70964
7/3/2005	0.490	4.20	-9.12	0.70996
7/3/2005	0.453	4.60	-8.97	0.71000
7/4/2005	0.454	6.60	-10.39	0.71113
7/4/2005	0.483	4.76	-9.10	0.70979
7/4/2005	0.522	5.18	-9.84	0.71040
7/4/2005	0.495	5.68	-9.37	0.70979
7/4/2005	0.525	5.45	-9.39	0.70980
7/6/2005	0.414	7.13	-9.31	0.71060
7/6/2005	0.417	3.78	-9.28	0.70970
7/6/2005	0.472	5.30	-8.79	0.70979
7/6/2005	0.502	6.31	-9.88	0.71086
7/7/2005	0.455	4.52	-9.27	0.70979
7/11/2005	0.483	5.00	-9.27	0.70989
7/11/2005	0.549	7.34	-9.64	0.71045
7/11/2005	0.447	6.82	-9.66	0.71102
7/11/2005	0.428	7.42	-10.03	0.71103
7/11/2005	0.454	3.10	-8.71	0.70943
7/11/2005	0.577	4.98	-9.46	0.71056
7/11/2005	0.453	3.93	-9.95	0.70970
7/11/2005	0.440	3.75	-9.92	0.70971
7/12/2005	0.483	3.46	-8.72	0.71054
7/12/2005	0.481	6.77	-9.53	0.71072
7/12/2005	0.566	6.36	-10.40	0.70987

Table A4.2 continued

Capture Date	Sr/Ca [mmol/mol]	Ba/Ca [μ mol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
7/12/2005	0.486	4.37	-9.52	0.71033
7/12/2005	0.570	6.01	-9.69	0.71016
7/12/2005	0.485	8.35	-10.01	0.71067
7/12/2005	0.421	5.68	-9.89	0.71119
7/12/2005	0.516	7.24	-9.10	0.70978
7/12/2005	0.496	5.67	-9.05	0.70986
7/12/2005	0.479	5.19	-9.13	0.71027
7/12/2005	0.415	5.44	-8.91	0.70983
7/12/2005	0.460	5.37	-9.42	0.70971
7/13/2005	0.526	4.83	-9.34	0.71035
7/13/2005	0.494	5.92	-9.41	0.71046
7/13/2005	0.617	7.20	-9.86	0.71032
7/13/2005	0.507	4.77	-10.80	0.71109
7/13/2005	0.769	6.18	-10.80	0.71061
7/14/2005	0.452	7.04	-9.09	0.70964
7/14/2005	0.508	6.06	-9.30	0.71034
7/14/2005	0.512	6.02	-9.87	0.71074
7/14/2005	0.473	7.04	-8.17	0.70926
7/14/2005	0.424	5.68	-9.13	0.71057
7/14/2005	0.424	6.26	-9.71	0.71019
7/14/2005	0.544	4.64	-9.76	0.71052
7/14/2005	0.553	11.33	-11.15	0.71055
7/14/2005	0.457	8.90	-9.80	0.71085
7/14/2005	0.432	6.45	-9.44	0.71108
7/14/2005	0.518	5.46	-9.46	0.70969
7/15/2005	0.487	5.06	-8.51	0.71017
7/15/2005	0.414	4.27	-8.64	0.70976
7/15/2005	0.493	7.92	-9.34	0.70991
7/15/2005	0.478	7.26	-10.03	0.71079
7/15/2005	0.475	5.32	-8.91	0.70988
7/15/2005	0.465	8.54	-10.31	0.71092
7/15/2005	0.497	4.85	-9.37	0.70945

Table A4.2 continued

Capture Date	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ ¹⁸ O VPDB	⁸⁷ Sr/ ⁸⁶ Sr
7/15/2005	0.493	6.88	-10.30	0.71086
7/15/2005	0.500	4.06	-9.17	0.70968
7/15/2005	0.581	5.18	-10.03	0.71023
7/15/2005	0.445	7.98	-9.48	0.71082
7/16/2005	0.383	7.19	-6.95	0.70910
7/16/2005	0.555	5.14	-7.97	0.70938
7/16/2005	0.494	6.64	-9.70	0.70977
7/16/2005	0.436	4.76	-7.96	0.71034
7/16/2005	0.552	7.40	-10.51	0.71088
7/16/2005	0.515	4.57	-9.17	0.70975
7/16/2005	0.516	5.51	-9.13	0.71052
7/16/2005	0.458	6.05	-9.36	0.71058
7/16/2005	0.529	3.64	-8.78	0.70993
7/16/2005	0.467	7.90	-8.92	0.70987
7/16/2005	0.504	5.27	-9.50	0.70995
7/16/2005	0.441	7.26	-9.24	0.70978
7/16/2005	0.485	7.83	-11.80	0.71132
7/16/2005	0.446	7.88	-9.21	0.70982
7/16/2005	0.448	5.30	-9.11	0.70989
7/16/2005	0.520	5.53	-10.40	0.71078
7/18/2005	0.510	6.48	-9.46	0.70992
7/18/2005	0.527	4.52	-8.64	0.71063
7/18/2005	0.471	6.14	-10.98	0.71099
7/18/2005	0.511	4.93	-9.18	0.71029
7/18/2005	0.584	5.73	-9.29	0.70985
7/18/2005	0.497	5.45	-9.22	0.70976
7/18/2005	0.594	8.07	-8.56	0.71123
7/18/2005	0.491	5.07	-8.54	0.71008
7/18/2005	0.485	4.55	-7.96	0.70978
7/18/2005	0.529	4.86	-8.65	0.70979
7/18/2005	0.631	8.73	-8.48	0.71028
7/18/2005	0.480	5.30	-9.21	0.71004

Table A4.2 continued

Capture Date	Sr/Ca [mmol/mol]	Ba/Ca [μ mol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
7/18/2005	0.507	10.60	-10.90	0.71104
7/18/2005	0.476	3.89	-9.24	0.70973
7/20/2005	0.464	6.29	-8.77	0.70981
7/20/2005	0.498	6.02	-11.39	0.71116
7/20/2005	0.420	7.49	-9.27	0.70980
7/20/2005	0.529	6.85	-9.40	0.70971
7/20/2005	0.530	6.75	-9.37	0.71028
7/20/2005	0.446	7.30	-10.11	0.71098
7/20/2005	0.488	5.99	-8.95	0.71019
7/20/2005	0.457	6.03	-9.44	0.70984
7/20/2005	0.570	5.87	-9.17	0.70987
7/20/2005	0.431	5.49	-9.75	0.71015
7/21/2005	0.429	5.32	-8.92	0.70967
7/21/2005	0.523	3.50	-8.59	0.70953
7/21/2005	0.494	6.34	-9.56	0.70979
7/21/2005	0.448	6.71	-9.41	0.70978
7/21/2005	0.550	6.10	-10.00	0.71134
7/21/2005	0.439	7.04	-8.88	0.70966
7/21/2005	0.472	6.52	-9.60	0.71064
7/22/2005	0.502	4.25	-10.36	0.70881
7/22/2005	0.481	8.57	-9.76	0.71070
7/22/2005	0.422	4.09	-9.98	0.70967
7/22/2005	0.471	4.81	-9.23	0.70966
7/22/2005	0.467	3.68	-9.30	0.70982
7/22/2005	0.526	5.89	-8.96	0.70972
7/22/2005	0.537	5.70	-9.30	0.71000
7/22/2005	0.580	6.63	-9.25	0.71046
7/22/2005	0.479	6.19	-10.65	0.71046
7/22/2005	0.480	5.01	-9.17	0.70970
7/22/2005	0.503	5.76	-8.90	0.70977
7/22/2005	0.449	7.57	-10.18	0.71115
7/22/2005	0.676	11.38	-9.54	0.71037

Table A4.2 continued

Capture Date	Sr/Ca [mmol/mol]	Ba/Ca [μ mol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
7/22/2005	0.609	4.43	-9.12	0.71027
7/22/2005	0.435	3.32	-7.74	0.70955
7/22/2005	0.473	8.11	-9.65	0.71104
7/22/2005	0.484	6.37	-8.78	0.71033
7/22/2005	0.429	3.84	-9.10	0.70963
7/22/2005	0.503	4.90	-9.20	0.70963
7/22/2005	0.654	6.60	-9.48	0.71053
7/22/2005	0.452	3.82	-8.81	0.70983
7/22/2005	0.482	4.90	-8.76	0.70978
7/22/2005	0.654	8.30	-8.88	0.70977
7/22/2005	0.471	4.69	-8.59	0.70981
7/22/2005	0.501	3.65	-10.20	0.70968
7/22/2005	0.538	6.27	-9.91	0.71116
7/22/2005	0.443	4.79	-9.31	0.70983
7/22/2005	0.566	6.77	-8.98	0.70976
7/22/2005	0.553	3.94	-9.40	0.71043
7/22/2005	0.535	4.45	-8.98	0.71023
7/22/2005	0.583	7.32	-9.01	0.70986
7/22/2005	0.465	5.21	-8.86	0.70971
7/22/2005	0.441	6.26	-10.26	0.71084
7/22/2005	0.455	5.22	-9.12	0.70971
7/22/2005	0.495	4.97	-9.05	0.70965
7/22/2005	0.533	5.72	-8.68	0.70992
7/22/2005	0.537	6.61	-8.00	0.71080
7/22/2005	0.474	7.24	-9.65	0.71091
7/22/2005	0.476	7.98	-10.96	0.71117
7/22/2005	0.470	5.92	-8.75	0.71021
7/22/2005	0.465	5.51	-9.25	0.70968
7/22/2005	0.540	4.95	-9.70	0.71053
7/22/2005	0.471	5.20	-9.08	0.70967
7/22/2005	0.519	5.13	-8.99	0.70968
7/22/2005	0.445	6.01	-8.62	0.71102

Table A4.2 continued

Capture Date	Sr/Ca [mmol/mol]	Ba/Ca [μ mol/mol]	$\delta^{18}\text{O}$ VPDB	$^{87}\text{Sr}/^{86}\text{Sr}$
7/22/2005	0.525	7.00	-9.32	0.70977
7/22/2005	0.483	7.90	-9.59	0.71122
7/22/2005	0.496	5.17	-9.35	0.70987
7/22/2005	0.633	13.12	-9.27	0.70976
7/22/2005	0.438	6.28	-8.62	0.71072
7/22/2005	0.653	11.98	-7.82	0.71108
7/22/2005	0.455	6.00	-10.00	0.71091
7/22/2005	0.437	6.73	-9.11	0.71104
7/22/2005	0.454	4.17	-9.24	0.70974
7/22/2005	0.481	8.10	-9.74	0.71131
7/22/2005	0.433	7.09	-8.64	0.71083
7/22/2005	0.452	6.67	-10.18	0.71088
7/22/2005	0.429	5.48	-9.29	0.71122
7/22/2005	0.522	7.67	-10.53	0.71075
7/22/2005	0.493	6.33	-8.91	0.71001
7/22/2005	0.546	5.68	-8.73	0.71032
7/22/2005	0.469	7.73	-9.95	0.71046
7/22/2005	0.521	5.17	-8.34	0.70968
7/22/2005	0.460	6.28	-9.04	0.71104
7/22/2005	0.472	4.41	-9.10	0.70969
7/22/2005	0.475	4.87	-7.79	0.71041
7/24/2005	0.444	5.26	-10.28	0.71096
7/24/2005	0.484	5.99	-9.19	0.70981
7/24/2005	0.499	7.12	-9.13	0.70989
7/24/2005	0.629	5.63	-9.85	0.71071
7/24/2005	0.468	5.04	-8.83	0.70982
7/24/2005	0.508	5.85	-9.49	0.71047
7/24/2005	0.524	4.44	-8.84	0.71006
7/24/2005	0.508	4.63	-9.33	0.70997
7/24/2005	0.528	5.68	-8.35	0.70975
7/24/2005	0.516	5.03	-8.42	0.71017
7/24/2005	0.499	6.84	-10.92	0.71086

Table A4.2 continued

Capture Date	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]	δ¹⁸O VPDB	⁸⁷Sr/⁸⁶Sr
7/24/2005	0.571	7.17	-9.05	0.70966
7/25/2005	0.491	3.11	-6.31	0.70857
7/25/2005	0.664	1.48	-5.24	0.70876
7/25/2005	0.520	8.09	-6.19	0.70882
7/25/2005	0.450	4.36	-9.48	0.70964
7/25/2005	0.587	8.99	-10.60	0.71072
7/25/2005	0.579	4.73	-10.28	0.70994
7/25/2005	0.496	5.29	-9.65	0.71004
7/25/2005	0.538	5.07	-9.03	0.71045
7/25/2005	0.532	8.55	-9.54	0.70989
7/25/2005	0.483	3.57	-8.40	0.71073
7/26/2005	0.773	2.53	-5.85	0.70894
7/26/2005	0.471	8.09	-10.43	0.71100
7/26/2005	0.433	4.20	-9.29	0.70991
7/26/2005	1.014	14.47	-10.35	0.70997
8/2/2005	0.473	5.01	-9.29	0.70969
8/2/2005	0.393	3.79	-9.33	0.70968

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16. Abstract (Limit: 200 words) In an assessment of river-specific signatures in American shad (<i>Alosa sapidissima</i>), stable isotope and elemental ratios in otoliths of juveniles produced accurate natal tags from 12 rivers. The database was expanded to include 20 rivers from Florida to Quebec, encompassing all major spawning populations. Regressions between otolith and water chemistry for those rivers where both were collected showed significant relationships for Sr:Ca, Ba:Ca, $\delta^{18}\text{O}$, and $^{87}\text{Sr}:^{86}\text{Sr}$ ratios but not for Mg:Ca or Mn:Ca. Cross-validated classification accuracies of known-origin juveniles averaged 93%. Adults returning to spawn in the York River were classified according to their otolith composition. Only 6% of spawners originated from rivers other than the York, supporting the hypothesis that most American shad spawn in their natal river. Of remaining spawners, 79% originated from the Mattaponi River and 21% from the Pamunkey River, suggesting less fidelity to individual tributaries. Otolith signatures were also used in mixed-stock analyses of immature migrants in the Gulf of Maine. Mixed-stock compositions were dominated by fish from the Shubenacadie and Hudson rivers, with an increasing proportion of Potomac River fish over time. In contrast to results from adult tagging studies, southern stocks were virtually absent. These data suggest ontogenetic shifts in migratory behavior.			
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